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**The influence of a patent ductus arteriosus on the  
peripheral muscle oxygenation and perfusion in  
neonates**

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# Cooperation

This thesis arose from a cooperation of the clinics in Munich and Graz:

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# 1 Introduction

## 1.1 Prematurity

### 1.1.1 Definitions

The world health organization (WHO) defines preterm as babies born alive before 37 weeks of pregnancy (less than 259 days) are completed.<sup>[1]</sup> This can be further subdivided into:<sup>[2]</sup>

- Extremely preterm (below 28 weeks of gestation)
- Very preterm (28 to less than 32 weeks of gestation)
- Moderate to late preterm (32 to less than 37 weeks of gestation)

Criteria for “born alive” are:<sup>[3]</sup>

- Heartbeat or
- Umbilical cord pulsation or
- Respiration or
- Voluntary movement

A preterm birth has to be differentiated from still birth and abort.

**Still birth** is a newborn which has a birth weight above 500g or  $\geq 22$  completed weeks of gestation, but does not show any of the vital signs mentioned above.<sup>[2, 3]</sup>

**Abort** is the expulsion of an embryo or fetus with a weight below 500g which does not show any of the vital signs mentioned above, independently of its gestational age.<sup>[3]</sup>

### 1.1.2 Epidemiology

In 2010, the average worldwide preterm birth rate was 11.1% (184 countries included), which totals to 14.9 million preterm neonates.<sup>[2]</sup> The preterm birth rate varies widely between countries, ranging from 18% in Malawi to 5% in several northern European countries. In 2010, 60% of the 14.9 million preterm neonates were born in Sub-Saharan Africa and South Asia.<sup>[2]</sup> In low-income countries preterm birth rates are highest. In contrary, high-income countries have the lowest preterm birth rates. However, high preterm birth rates can also be found in certain high-income countries like the USA (12.9%) and Austria (10.9%).<sup>[2]</sup>

In most of the countries studied, the preterm birth rate was higher in 2010 than in 1990.<sup>[2]</sup> This results partly from a registration increase of extremely preterm neonates. The increase in the number of moderate to late preterm can partly be explained by an increase in multiples, which tend to have a higher probability to result in preterm delivery.<sup>[4]</sup> The advances in assisted reproduction techniques account for more than 40% of multiple pregnancies.<sup>[4]</sup>

After pneumonia, preterm birth complications are the second largest direct cause of deaths in children under five years of age. Preterm birth complications are responsible for around 35% of the world's neonatal deaths per year.<sup>[2]</sup> Great variation in survival rates can be seen between high-income and low-income countries. At the moment, the rate of survival for neonates born in high-income countries before 28 weeks of gestation is more than 90%, whereas in low-income countries it is less than 10%.<sup>[2]</sup> Most of the worldwide preterm neonates are in the group of moderate to late preterm (84%). Most of these neonates do not need intensive care, they can survive with supportive care, which is not available in most low-income countries.<sup>[2]</sup>

Due to the advances in intensive care, high survival rates have been reached in the last years. In the mid-1960s extremely low birth weight infants (ELBW; birth weight below 1000g) had a mortality rate of 95%.<sup>[4]</sup> In 2000, the survival rate of neonates with birthweight between 900-1000g was 95%. Today, more than 50% of neonates, born after 24 weeks of gestation, survive.<sup>[4]</sup> Today, the limit of viability is between 23 and 25 weeks of gestation.<sup>[5]</sup>

The downside to the decrease in mortality is the increase in morbidity. In the last years the mortality decreased, but the morbidity stayed approximately the same. Especially ELBW infants are facing high risks of developing complications.<sup>[4]</sup> Neonates born after 23-24 weeks of gestation have a risk of 20% to develop major disabilities. In addition, mild disabilities occur in up to 30% of the extremely preterm neonates. Therefore, in up to 50% of the surviving neonates long-term disabilities occur.<sup>[4]</sup>

### **1.1.3 Morbidity**

In the last decades, mortality rate of preterm neonates has improved significantly. In addition, the gestational age-specific mortality rate has also improved during this period.<sup>[6]</sup> However, preterm infants are still vulnerable to many complications. As mentioned above, the risk of a preterm newborn to develop serious morbidity depends



on the gestational age. Neonates born extremely preterm have the highest rates of complications.<sup>[6]</sup>

The problems of preterm neonates arise from the immature organ systems which are not prepared for extra uterine life yet.<sup>[6]</sup>

In the following section, the most common morbidities in the care of preterm neonates are listed.

#### **a. Respiratory system**

Surfactant, which is produced by the lungs by around 30 to 32 weeks of gestation, helps to keep the alveoli open. Infants born before Surfactant is produced are of high risk of developing a respiratory distress syndrome (RDS).<sup>[6]</sup> The lungs of these infants have atelectasis and are under-ventilated, resulting in an under-supply of oxygen. Neonates developing a RDS become symptomatic with lung specific symptoms like tachypnea, grunting, diminished breath sounds and difficulty in maintaining sufficient work of breathing.<sup>[6]</sup> Unspecific symptoms, e.g. poor skin color, accompany the respiratory symptoms. This can lead to respiratory failure due to fatigue, apnea or hypoxia. In the last years, women who are at risk for preterm delivery receive glucocorticoids to reduce the risk of RDS. Since this treatment, the incidence and severity of RDS has been reduced.<sup>[6]</sup>

RDS can lead to the chronic disorder of bronchopulmonary dysplasia (BPD). The main reasons for BPD are an immature lung, inflammation, baro traumata, and toxic effects of oxygen.<sup>[6]</sup> Main risk factors are extreme prematurity (below 28 weeks of gestation), respiratory distress with positive-pressure ventilation, high oxygen concentrations, and a patent ductus arteriosus (PDA).<sup>[6]</sup>

Apnea is another commonly seen problem in preterm neonates. Infants with apnea cease breathing for at least 20 seconds which often coincides with bradycardia. This is mainly due to the immaturity of the control of breathing. They respond quickly to stimulation, but need constant monitoring. Apnea generally resolves as the preterm neonate matures.<sup>[6]</sup>

#### **b. Gastrointestinal system**

Necrotizing enterocolitis (NEC) is an acute inflammation of the small or large intestine and can lead to perforation of the intestines.<sup>[6]</sup> Again, extremely

preterm infants face higher risks of developing NEC. More specifically, 3% of neonates born before 33 weeks of gestation and 7 percent of neonates with a birth weight under 1500g face the above mentioned disease.<sup>[7-9]</sup> The exact cause is unknown, but it is multifactorial, as usual in many diseases of preterm neonates. Risk factors for a vulnerable intestine are prematurity and reduced oxygen supply caused by placenta insufficiency, hypovolemia, anemia, and a PDA.<sup>[10]</sup> For example enteral feeding and oral medication can stress the already vulnerable intestine, resulting in damage of the mucosa and in superinfection.<sup>[10]</sup> Survivors experience severe short- and long-term morbidities. Permanent colostomy or ileostomy is a common long-term morbidity.<sup>[6]</sup> Another factor commonly influencing the quality of life is the need of repeated surgical procedures. Furthermore, liver failure, prolonged parenteral feeding, and malabsorption syndromes are likely to occur.<sup>[6]</sup>

**c. Cardio-vascular system**

Preterm neonates tend to suffer from a severe hypotension shortly after delivery. The cause is multifactorial, but an immature regulation of the peripheral resistance, followed by a vasodilation of vessels is the main reason for hypotension post partum. Around 20-30% of neonates with a birth weight of less than 1500g develop a symptomatic PDA, resulting in an under supply with oxygen in peripheral tissues – the main subject of this thesis.<sup>[10]</sup>

**d. Central nervous system**

Behrman et al.<sup>[6]</sup> summarize: Up to 20% of neonates born with a body weight below 1500g suffer from intraventricular (IVH) or periventricular hemorrhage (PVH). Preterm neonates are not capable of adequate autoregulation of cerebral blood flow: their blood flow largely depends on their blood pressure. Hypoxia, ischemia and inflammation have an influence on the development of IVH and PVH.

Periventricular Leukomalacia (PVL) can be a complication of brain bleeds, but can also develop in the course of infection. PVL is the most common cause of neurodevelopmental disabilities. The risk for developing cerebral palsy is higher for children with PVL (around 10%), and the severity of cerebral palsy usually

correlates with the extend of the PVL. Mild spastic diplegia is the most common form of it.<sup>[6]</sup>

**e. Retinopathy of prematurity (ROP)**

Retinopathy of prematurity (ROP) is caused by obliteration of existing retinal blood vessels and retinal neovascularization. It can result in scarring of the retina and blindness. The incidence of ROP varies when comparing different studies. In a prospective study from Sweden, 73% of preterm neonates with gestational ages below 27 weeks of gestation showed ROP (at any stage) and severe ROP in 35%.<sup>[11]</sup> In comparison, in a study conducted in Austria severe ROP was reported in 16% of babies with a gestational age of less than 27 weeks at birth.<sup>[12]</sup> This variation may be partly accounted for by the difference in the proportion of infants at high risk of ROP who survive when born at early gestational ages.<sup>[13]</sup> High doses of oxygen are the main cause of ROP, which is a disease only occurring in preterm neonates. The contribution of oxygen to the development of ROP is unquestioned, but it is a complex disorder which is still subject of research.<sup>[13]</sup>

**f. Infections**

Infections are a common complication in neonatal care. 48% of all infections occur in infants during the first year of life, and more than 27% occur in the first four weeks of life. Throughout the world around 1.6 million neonates die from infections every year.<sup>[14-17]</sup>

The inability of responding adequately because of immunologic deficiencies, the difficulty in diagnosis, and the increasing survival of more and more preterm infants, contribute to the high morbidity and mortality due to infection.

The diagnosis of infection still is a big challenge in neonatal care. Early signs and symptoms are often hard to detect or may be misinterpreted. The clinical course of an infection may be fulminant and can lead to disseminated intravascular coagulation (DIC), septic shock, and death shortly after the onset of clinical manifestation.<sup>[18]</sup>

Since the diagnosis of an infection or sepsis is difficult and often too late, new methods are being examined for earlier detection of infections. Near-infrared

spectroscopy (NIRS) is one of these promising methods for detecting changes in the peripheral microcirculation earlier than currently possible with common methods.

## 1.2 Ductus arteriosus

### 1.2.1 Physiological background

Since fetal blood is oxygenated in the placenta and not in the lungs, lung perfusion during fetal life is not necessary. Special anatomic conditions cause the fetal circulatory system to work differently than in a newborn baby. Guyton A.C. and Hall J.E. summarize:<sup>[19]</sup>

*“First, blood returning from the placenta through the umbilical vein passes through the ductus venosus, mainly bypassing the liver. Then most of the blood entering the right atrium from the inferior vena cava is directed in a straight pathway across the posterior aspect of the right atrium and through the foramen ovale directly into the left atrium. Thus, the well-oxygenated blood from the placenta enters mainly the left side of the heart, rather than the right side, and is pumped by the left ventricle mainly into the arteries of the head and forelimbs. The blood entering the right atrium from the superior vena cava is directed downward through the tricuspid valve into the right ventricle. This blood is mainly deoxygenated blood from the head region of the fetus, and it is pumped by the right ventricle into the pulmonary artery and then mainly through the ductus arteriosus into the descending aorta, then through the two umbilical arteries into the placenta, where the deoxygenated blood becomes oxygenated.”*

Since the lung of a fetus does not have the task of oxygenating blood alveoli as well as lung vessels are collapsed. Thus, there is a very high resistance in the lungs resulting in a very high pulmonary pressure. Because of the low resistance in the aorta and the large vessels of the placenta, there is a very low pressure in the aorta.<sup>[19]</sup> In fetal circulation, the pressure in the pulmonary artery is usually higher than the pressure in the aorta. As a result, almost all the blood in the pulmonary artery passes through the DA into the descending aorta, bypassing the lungs. This is equivalent to a right-to-left shunt. Because of the DA, only 12% of all blood flows through the lungs; after birth this changes drastically.<sup>[19]</sup>

Immediately after birth, the baby begins to breathe and the lungs inflate. The alveoli fill with air and the pulmonary blood vessel resistance decreases tremendously. Whenever the cord is clamped this results not only in the stop of blood flow, but also segregates the low-pressure circuit of the placenta from the neonatal circulation.<sup>[19]</sup> As a result, systemic and thus aortic pressure rises. This causes a change in direction of blood flow

via the DA, now going from the aorta into to the pulmonary artery, equivalent to a left-to-right shunt. From a circulatory point of view this is important, because this left-to-right shunt via the DA compensates for the loss of preload due to cord clamping. Because of the pressure changes in the two atria, the foramen ovale closes.<sup>[19]</sup>

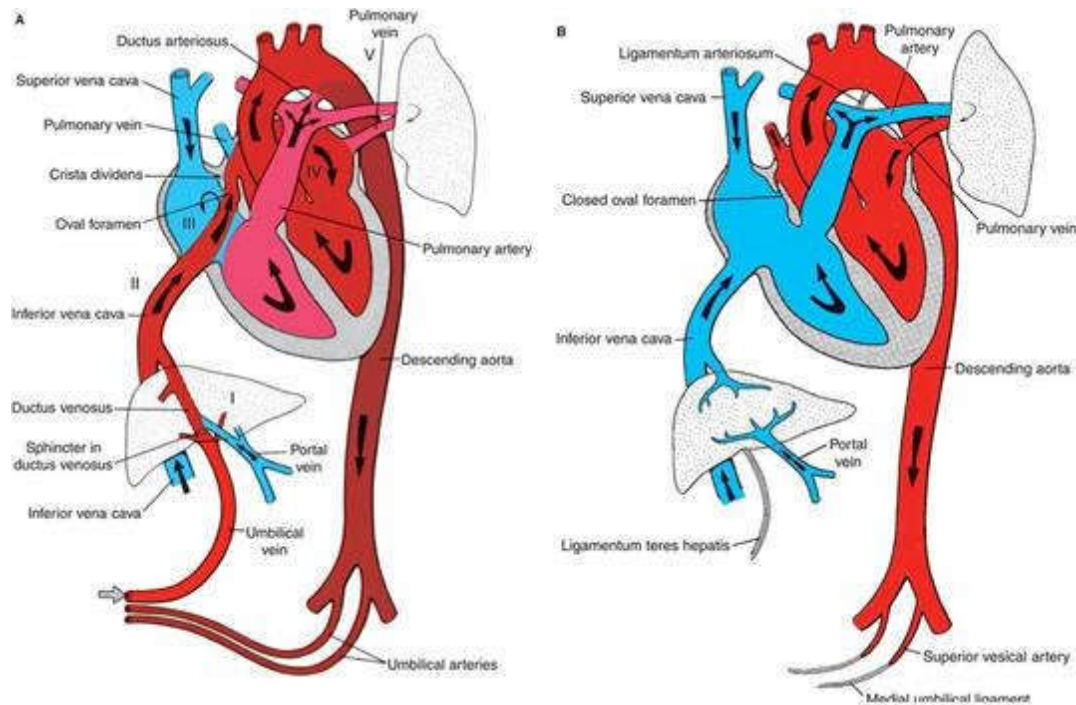


Figure 1: Fetal circulation (A), Neonatal circulation (B)<sup>[20]</sup>

### 1.2.2 Ductus arteriosus closure

The DA closure usually takes place in two phases. Primarily, there is the “functional” closure within the first hours after birth, followed by the “anatomical” closure over the next several days.

The functional closure of the DA is an active process which is based on a constriction of the smooth muscles in the DA. The various factors that are involved in this process:<sup>[21]</sup>

- Since the resistance in the pulmonary vessels decreases after birth, there is a decrease in blood pressure over the DA.<sup>[21]</sup>
- The postnatal increase in arterial SaO<sub>2</sub> causes the smooth muscles in the DA to contract. A cytochrome P<sub>450</sub> hemoprotein in the plasma membrane of the smooth muscle seems to play an important role of changes in the ductus triggered by

oxygen.<sup>[22, 23]</sup> By inhibiting potassium channels, oxygen causes the smooth muscle cell membrane to depolarize. Furthermore, there is an increase in intracellular calcium and in the production of Endothelin-1 (a strong vasoconstrictor).<sup>[24-27]</sup> However, the exact mechanisms of the change of the membrane potential and the exact role of Endothelin-1 are still unclear.<sup>[23, 28]</sup>

- Prostaglandins (PG), produced by the DA, are strong vasodilators that oppose constriction of the DA during gestation.<sup>[29]</sup> Especially Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), which is the strongest vasodilator among the PGs produced by the ductus, plays an important role in maintaining the patency of the DA during fetal life.<sup>[30]</sup> Both isoforms of the cyclooxygenase (COX-1, COX-2), which is the enzyme responsible for PG synthesis, are produced in the fetal DA.<sup>[31]</sup> The DA is also influenced by circulating PGE<sub>2</sub>, which seems to originate from the placenta.<sup>[32]</sup> In adults, PGE<sub>2</sub> is eliminated by the lungs. In a fetus, the lungs are not capable of eliminating PGE<sub>2</sub> which results in high fetal PGE<sub>2</sub> concentrations.<sup>[33]</sup> After birth, circulating PGE<sub>2</sub> decreases (there is no more placental PG production and increase in pulmonary elimination of PG) and the sensibility to PG decreases as well (reduction of the number of receptors).<sup>[34]</sup> These mechanisms result in a closure of the DA.
- Nitric oxide (NO) is produced by the DA in utero and plays an important role in keeping the DA open during fetal life.<sup>[29]</sup> In contrast to PGE<sub>2</sub>, the DA remains sensible to NO after delivery.

The initial functional closure of the DA is followed by the anatomical closure. The thickness of the DA wall correlates with the gestational age. After reaching a certain thickness the DA wall requires intramural vasa vasorum. After delivery, the DA constricts and vasa vasorum blood flow to the outer muscle media ceases resulting in an under-supply of the smooth muscles.<sup>[21]</sup> This hypoxic zone induces smooth muscle death in the media, prevents production of PGE<sub>2</sub> and NO in the DA, and induces production of hypoxia-induced growth factors (TGF- $\beta$  and vascular endothelial growth factor (VEGF)).<sup>[35]</sup> This stimulates endothelial proliferation, leading to extensive neointimal thickening. These mechanisms lead to fibrosis and a permanent closure of the DA, producing a structure called the ligamentum arteriosum.<sup>[21, 36]</sup>

### **1.2.3 Patent ductus arteriosus**

In term neonates, closure of the DA usually occurs within 24 hours after birth.<sup>[36]</sup> The incidence of a patent ductus arteriosus (PDA) is around 1 of 2000 births in term neonates, accounting for around 5-10% of all congenital heart diseases. In preterm babies, a PDA is a lot more common. It has been shown that in infants born with a birth weight of less than 1000g, the DA remains open in 66% beyond the first week of life.<sup>[37]</sup> The probability of a DA to remain open beyond 24h after birth decreases with increasing gestational age and weight. Various factors have been found which influence the closure of the DA in preterm neonates.<sup>[38]</sup>

According to Clyman<sup>[21]</sup>, closure of the preterm DA differs from the closure of the term DA: After birth, a preterm DA has a very high sensitivity to PGE<sub>2</sub>. This is the most important mechanism that prevents the DA from constricting. In addition to the higher sensitivity for PGE<sub>2</sub>, the premature DA produces more NO after birth. Even if the DA contracts, it often fails to develop sufficient hypoxemia and therefore anatomic remodeling does not occur. A preterm DA requires higher degree of contraction to result in a hypoxia comparable to the hypoxia in term neonates. The DA wall of preterm babies is thinner compared to term babies and therefore vasa vasorum do not exist. Without intramural vasa vasorum the DA is not able to rapidly raise the distance of diffusion across its wall during the process of constriction after birth. The preterm DA needs absolute cessation of luminal flow in order to be able to result in the same hypoxia as expressed in term neonates. If the preterm DA cannot develop the hypoxia needed for anatomic closure, death of smooth muscle tissue, and remodeling it remains open or can easily reopen.

### **1.2.4 Systemic consequences of a patent ductus arteriosus**

The severity of a PDA depends on its hemodynamic influence on the systemic circulation. The clinical history may vary from completely asymptomatic to congestive heart failure or Eisenmenger's syndrome.<sup>[36]</sup> The left-to-right shunt over the PDA results in pulmonary overcirculation and systemic hypoperfusion. Studies have shown that, despite an excessive left-to-right shunt, neonates are capable of increasing their left ventricular output in order to maintain an effective systemic blood flow. Only with left-to-right shunts of more than 50% of left ventricular output, effective systemic blood flow decreases.<sup>[21, 39, 40]</sup> The preterm neonate is able to preserve sufficient cerebral blood



flow, but cannot provide sufficient blood flow to post ductal organs. After closure of the PDA blood flow to post ductal organs returns to normal.<sup>[40]</sup>

Despite the ability of increasing the left ventricular output when a PDA is present, blood flow distribution changes significantly. This effect even occurs in neonates with a small left-to-right shunt over the PDA.<sup>[39]</sup> This redistribution of blood flow most likely influences perfusion of organs and peripheral tissues. Perfusion of the following organs is most likely to be affected: Bone, skin, skeletal muscle, gastrointestinal tract, and kidneys.<sup>[21]</sup> These organs may suffer of severe hypoperfusion even before an influence on the left ventricular function is noted. Hypoperfusion of peripheral organs can influence the development of the most common comorbidities of preterm neonates.<sup>[40]</sup>

The pulmonary overcirculation may cause the lungs to develop pulmonary congestion, pulmonary edema, and respiratory difficulties. Benitz WE.<sup>[41]</sup> summarizes that prolonged patency of a DA may be a cause of many diseases of a neonate like prolonged assisted ventilation, pulmonary hemorrhage, bronchopulmonary dysplasia, necrotizing enterocolitis, cerebral diseases (IVH, PVL, cerebral palsy), and failure of renal function.

It is not yet clear, to what extent the incidence and severity of the mentioned complications depends on the hemodynamic changes of the PDA. However, attempts to close a PDA through medication or surgery have been made to reduce prematurity complications.<sup>[42]</sup>

### **1.2.5 Diagnosis of a patent ductus arteriosus**

The hemodynamic effects of large left-to-right shunting PDA may be seen clinically, in echocardiography, or in serum biomarkers. Gournay<sup>[36]</sup>, Benitz<sup>[42]</sup>, and Schneider<sup>[43]</sup> summarize:

- Clinical manifestations may include a typical continuous heart murmur, located in the second intercostal space near the sternum on the left side. This heart murmur is referred to as “machinery” murmur. Tachycardia (>170 bpm), prominent arterial pulses, low blood pressure or low diastolic blood pressure with a widened pulse pressure can be symptoms of a hemodynamically relevant PDA. Respiratory findings, increasing demand of oxygen, and inability to reduce ventilation may suggest a large ductal shunt. Nevertheless, clinical symptoms are unspecific.<sup>[36, 42]</sup>

- Doppler echocardiography is the procedure of choice for evaluating the DA. It is a very sensitive technique that can evaluate the diameter, the shunt behavior and associated lesions. Echocardiography will be discussed in chapter 1.5.<sup>[36]</sup>
- An X-ray of the thorax can be done to show cardiac and pulmonary lesions, like cardiomegalopathy or increased pulmonary vascular markings.<sup>[43]</sup>
- In patients with a large and/or long persisting PDA, the electrocardiogram (ECG) may show symptoms of chronic volume overload. This includes left ventricular hypertrophy, enlargement of the left atrium, tachycardia, and atrial fibrillation. Furthermore, signs of right atrial enlargement and hypertrophy of the ventricles can be found.<sup>[43]</sup>
- Myocytes located in atria and ventricles synthesize brain natriuretic peptide (BNP). They react to high pressure and volume strain with a higher production of BNP. Nuntarumit et al.<sup>[44]</sup> have shown that N-terminal probrain natriuretic peptide (NT-proBNP) is a sensitive marker for assessing the hemodynamic relevance of a PDA in preterm neonates. It correlates well with echocardiographic measures of the DA shunt. Successful closure of the PDA corresponded to a decline in plasma NT-proBNP.<sup>[44]</sup>

The assessment whether a PDA is hemodynamically significant or not can be made by clinical examination, the measurement of biomarkers in the serum or echocardiography. As mentioned above, the clinical symptoms are not specific and do not correlate well with echocardiographic findings. The Doppler echocardiography permits confirmation of DA patency as well as ductus dimensions and shunt behavior.<sup>[42]</sup> Substantial ductal shunting is often associated with:<sup>[45, 46]</sup>

- a ratio above 1.5:1 of left atrial to aortic root dimension
- a ductal diameter larger than 1.5 mm/kg bodyweight
- left ventricular volume and pressure load
- reverse diastolic blood flow in the aorta descendens or in the renal or cerebral arteries

### **1.2.6 Treatment of a patent ductus arteriosus**

Until now, the standard therapy for preterm infants with PDA has been the closure of the DA. The first attempt to close the PDA usually is the stimulation with

cyclooxygenase-inhibitors (COX-inhibitor) like Ibuprofen or Indomethacin. If conservative therapy with COX-inhibitors fails, surgical ligation is the next step.

- Pharmacologic treatment with COX-Inhibitors is the primary attempt for closure of the PDA. COX-inhibitors inhibit the enzyme necessary for prostaglandin production. As prostaglandins are essential in keeping the DA open during fetal life, inhibition of prostaglandins supports closure of the DA.<sup>[31]</sup> The DA of preterm neonates has a very high sensitivity for PG and therefore has the tendency for patency of the DA: therefore COX-Inhibitors are the first line treatment in PDA. Indomethacin and Ibuprofen are the most commonly used prostaglandin inhibitors. However, since Indomethacin is a very strong vasoconstrictor, studies have found an association with application of Indomethacin and the appearance of NEC, renal impairment, brain white matter injury, intestinal perforation, and platelet dysfunction.<sup>[47-50]</sup> Therefore, Ibuprofen was later introduced; it is said to have less vasoconstrictive effects on end-organ microcirculation.<sup>[51]</sup> Nevertheless, it has also been associated with renal effects, pulmonary hypertension, and hyperbilirubinemia.<sup>[52, 53]</sup> Studies have been conducted to compare Ibuprofen- with Indomethacin-treatment and have shown that Ibuprofen was much safer in terms of incidence of NEC and oliguria, without any difference in efficiency.<sup>[51]</sup> Recently, Acetaminophen (Paracetamol) has been used for DA closure. Studies could not show any differences between Acetaminophen and Ibuprofen in efficiency.<sup>[54]</sup>
- If pharmacological treatment is not successful, surgical ligation is the next step taken in the process of treating DA patency: The surgery consists of a small left thoracotomy and the closure of the DA with a metal clip or ligation. For this surgery, no pulmonary bypass is needed. Possible complications that can occur are: pneumothorax, bleeding, and recurrent laryngeal nerve palsy. In general, complications are very rare and surgical ligation is well tolerated by the neonate.<sup>[36]</sup> After surgery, improvement of lung compliance has been found.<sup>[55]</sup> Nevertheless, a serious hypotension and a temporary dysfunction of the left ventricle can occur within 24 h after ligation.<sup>[56-58]</sup> Additionally, reduction in cerebral blood flow has been described postoperatively, especially in extremely preterm neonates.<sup>[59]</sup> It is highly recommended to weigh the benefit to risk ratio of surgical ligation thoroughly for each individual patient.<sup>[36]</sup>

The treatment of a PDA is still a very controversially discussed topic. When a PDA should be treated is one of the most controversially debated issues of PDA closure. In the last years, changes in the treatment of PDA towards “less treatment” have been made. There is no evidence that these changes bring advantages in the treatment.<sup>[37]</sup>

Different strategies for the use of pharmacological treatment have been investigated:

- In low gestational ages, prophylactic treatment (treatment on day 1, infants without symptoms) shows significant increase in efficacy and reduction in the rate of complications. Symptomatic PDA, PVL, PVH, and pulmonary complications are rare and also secondary surgical ligation can be avoided.<sup>[60, 61]</sup> Furthermore, prophylactic treatment does not have significant negative side effects. However, benefits for late morbidity, mortality, and neurosensory impairment have not been proven.<sup>[61]</sup> Because of 30-40% of the neonates being exposed to unnecessary prostaglandin inhibitor treatment, prophylactic medical treatment cannot be supported. Another study has shown that prophylactic treatment does not improve the survival rate without cerebral impairment, even though it decreases the rate of PVH and IVH.<sup>[62]</sup> However, the medical treatment of a PDA has to be considered for individuals at high risk of developing cerebral or other frequent complications.<sup>[63]</sup>
- Treatment of neonates with an echocardiographic diagnosed PDA before symptoms develop is defined as early asymptomatic treatment. A meta-analysis of three studies has shown that early asymptomatic treatment can reduce the risk of developing symptoms and that the oxygen dependency, especially in extremely preterm neonates, can be reduced.<sup>[63, 64]</sup>
- Two studies published in 2012 showed that moderately delayed PDA closure (11-14 days post-partum) significantly decreases the need of prostaglandin inhibitors or surgical PDA closure.<sup>[65, 66]</sup> However, an increase in chronic lung disease and death was found in one group of the trial. It is difficult to draw a conclusion out of the recent studies on this topic. It is possible that a moderately delayed treatment plan may be tolerated by stable, elder neonates, whereas the long term results for fragile neonates with symptomatic PDA are not known yet.<sup>[63]</sup> The authors of these studies state that further, larger studies have to be conducted to ensure the safety of this treatment approach.<sup>[65, 66]</sup>

## 1.3 Microcirculation

### 1.3.1 Definitions

Microcirculation is the circulation of blood in the smallest vessels of the blood system. Microcirculation is a broad term which includes terminal arterioles, capillaries, and venules.<sup>[67]</sup> Capillary exchange plays the main and most important role in the task of the blood stream in delivering oxygen and nutrients to peripheral tissues as well as to remove carbon dioxide (CO<sub>2</sub>). Furthermore, it has the task to deliver nutrients (like glucose, amino acids) as well as hormones and therapeutic drugs to the peripheral tissues.<sup>[67]</sup> In calm state, the area of the capillaries and post capillary venules sums up to around 300 m<sup>2</sup>, and during maximal perfusion up to 1000 m<sup>2</sup>.<sup>[68]</sup> The organization of the terminal vessels in different organs of the human body underlies great differences depending on the demands of the certain tissue.<sup>[67]</sup> In skeletal muscle or skin the perfusion in resting state is very low, and can increase considerably depending on the demand. In other organs like the heart the capillary bed is almost completely perfused all the time.<sup>[69]</sup> The number of perfused capillaries mainly depends on the tonus and therefore the diameter of the terminal arterioles. The exact mechanism of the regulation of the perfusion in the capillary bed will be discussed later in this chapter.

Depending on the requirements of the various sections of the microcirculation, the vessels have different characteristics:<sup>[19, 69, 70]</sup>

The arteries can be split into elastic arteries, muscular arteries, and arterioles. The central arteries (e.g. Aorta, Truncus pulmonalis, etc.) belong to the elastic arteries, since they have to accommodate huge amounts of blood which the heart ejects.<sup>[69]</sup> The subsequent arteries (e.g. Aa. brachialis, facialis, femoralis as well as the no-named smallest arteries) belong to the muscular type.<sup>[69]</sup> They have a thick layer of smooth muscle to control the blood flow through the capillary beds by contracting or dilating the size of the lumen. Vessels with an inner diameter of less than 100 to 300 µm and only one to two layers of smooth muscle are called arterioles. The diameter of the arterioles as well as the diameter of the smallest arteries determines the peripheral resistance. The metarterioles (the terminal arterioles) have smooth muscle fibers which enclose the vessel punctually, but do not have a continuous layer of smooth muscle. A precapillary sphincter is located where a capillary branches off a metarteriole.<sup>[19]</sup>

	Artery	Arteriole	Capillary	Venule	Vein
<b>Mean diameter</b>	4.0 mm	30.0 $\mu\text{m}$	8.0 $\mu\text{m}$	20.0 $\mu\text{m}$	5.0 mm
<b>Mean wall thickness</b>	1.0 mm	6.0 $\mu\text{m}$	0.5 $\mu\text{m}$	1.0 $\mu\text{m}$	0.5 mm

**Table 1: Properties of the different types of vessels**

Capillaries have a single layer of flattened endothelial cells with pericytes associated to them. There are no muscular or adventitial layers. Normal capillaries have a diameter of 7  $\mu\text{m}$ , but some can also be up to 40  $\mu\text{m}$  in diameter. These bigger capillaries are called sinusoids and are mainly found in the liver, lymph nodes, spleen, bone marrow and in glands of the endocrine system. The endothelial cells are bound together by tight junctions, gap junctions, or adherens junctions.<sup>[69]</sup> Capillaries can be continuous, fenestrated, or discontinuous. Capillaries of the continuous type are mainly found in the heart- and skeletal-muscle, as well as in skin, lung, and the central nervous system (CNS). In tissues with high molecular exchange such as the endocrine glands, the small intestine, and the kidneys, fenestrated capillaries can be found. Discontinuous capillaries are mainly found in the liver. The larger pores in this type of capillaries allow larger particles like proteins or blood cells to pass through.<sup>[19, 69]</sup>

Blood flows from the capillaries into venules. The smallest venules are similar to capillaries, only distinguishable by their convergent pattern of blood flow. The walls of larger venules contain smooth muscle. From venules, blood flows into veins that become larger in diameter the nearer to the heart. The largest veins, the venae cavae, empty into the right atrium.<sup>[70]</sup>

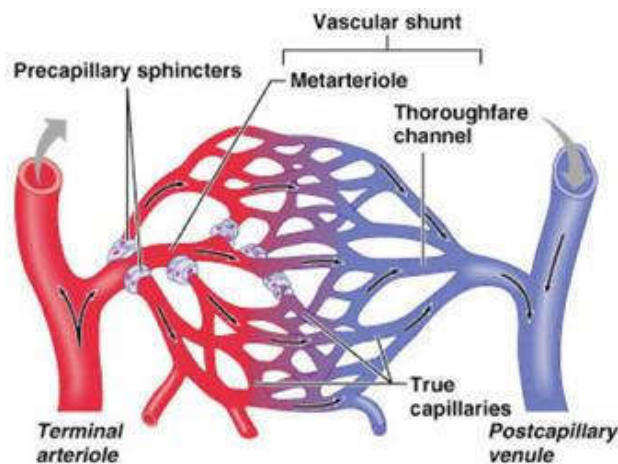


Figure 2: Schematic representation of the microcirculation<sup>[71]</sup>

### 1.3.2 Regulation of blood flow microcirculation

Adequate blood flow in tissues is maintained by complex mechanisms on systemic and regional levels. Metabolic and myogenic auto-regulatory mechanisms interact to maintain optimal tissue oxygenation.<sup>[19]</sup>

Blood does not flow continuously through the capillary bed. Vasomotion causes an alternating on and off flow and results from a contraction of metarterioles and capillary sphincters. The most important factor of the regulation of capillary blood flow is the concentration of oxygen in the tissue.<sup>[19]</sup>

The state of the smallest arterial vessels (arterioles and metarterioles) and precapillary sphincters can change rapidly from vasoconstriction to vasodilation in order to maintain adequate local blood flow.<sup>[19]</sup> If the rate of metabolism increases or the availability of nutrients and oxygen in a tissue decreases, vasodilatory substances are emitted. By diffusion, the vasodilatory substances reach the arterioles, metarterioles and precapillary sphincters. Vasodilatory substances involved in the dilation of vessels are histamine, phosphate compounds, adenosine, hydrogen, and potassium.<sup>[19]</sup> The most important local vasodilator of those mentioned above is adenosine. The nutrient lack theory states that oxygen and other nutrients can cause a contraction of vascular muscles. Therefore, a lack of oxygen causes the blood vessels to relax and dilate automatically.<sup>[19]</sup>

The autoregulation of blood flow in tissues is the ability of keeping the blood flow in tissues nearly constant, despite changes in systemic arterial blood pressure. It occurs

especially in the brain, heart, and the kidneys. Between an arterial pressure of 75 and 175 mmHg, tissues have the ability of autoregulation.<sup>[19]</sup>

There are long-term mechanisms that adapt the blood flow in a tissue to its needs. If metabolic demands of a tissue change over a longer period of time, e.g. less oxygen saturation of the air (higher altitude) or chronically higher nutrient demands, the nutrient supply has to adapt to match the needs of a tissue.<sup>[19]</sup>

Principally, long-term blood flow is controlled by vascularization. There is a physical reconstruction of the tissue vascularization to provide adequate supply of the tissue. The time required for a long-term regulation varies between tissues and age. In neonates it may only take a few days whereas in elderly people it may need months. Other examples for rapid change are fast growing tissues like cancer or scar tissue. The main factors involved in the formation of new blood vessels are oxygen, VEGF, angiogenin, and fibroblast growth factor. When a vessel is blocked, collaterals develop to enlarge the vascularization and maintain adequate blood flow.<sup>[19]</sup>

Additionally, tissue blood flow is controlled by hormones and ions. Norepinephrine (Noradrenaline) and epinephrine (Adrenaline) are vasoconstrictor hormones. Norepinephrine is a very powerful vasoconstrictor, whereas epinephrine is weaker and can even act as a vasodilator in certain tissues, e.g. coronary arteries. Both are secreted when the sympathetic nervous system is stimulated. Angiotensin II is another very strong vasoconstrictor. It mainly acts on the arterioles and increases the peripheral resistance. In addition to the water reabsorption in the kidneys, vasopressin is a very potent vasoconstricting substance. The stimulus for emission of endothelin is damage to the endothelium of a vessel and causes strong local vasoconstriction.<sup>[19]</sup>

Vasodilation is mainly caused by kinins and histamine. Strong arteriolar dilation and an increase in capillary permeability are caused by Bradykinin. Histamine is released by mast cells and basophil granulocytes in damaged tissue. It is another powerful vasodilator and increases capillary permeability, allowing plasma proteins and fluid to pass through into the tissue.<sup>[19]</sup>

### **1.3.3 Measurement techniques of microcirculation**

In the pathogenesis of organ failure in critically ill patients, microcirculation plays an important role. Especially in patients with sepsis, changes in microcirculation can be



found. New methods with the aim of visualizing microcirculation have been introduced in recent years.

The most commonly used clinical method is the **capillary refill time** measurement. Studies have shown that the capillary refill time is a good parameter for analyzing skin perfusion and consequently peripheral microcirculation.<sup>[72, 73]</sup> The downside to this simple non-invasive bedside technique is the influence of many different factors like age, ambient and skin temperature, site of measurement, amount and time of pressure, and the great inter-observer variation.<sup>[74]</sup>

Allen et al.<sup>[75]</sup> summarize two of the techniques used: In **Laser Doppler Fluxmetry (LDF)** monochromatic laser light (633 nm helium neon gas laser or a single mode 670 or 780 nm laser diode) is emitted into a tissue and scattered by moving erythrocytes. The frequency-broadened laser light is detected and the signal is processed. Single point measurements are of limited use, since blood flow in the skin has a high spatial variability. This limitation can be overcome with **Laser Doppler Perfusion Imaging (LDPI)**, in which a laser beam scans a certain area of the tissue to map the perfusion of this region of interest. The 2D color-coded LDPI images represent the blood flow of the area. The technique has not yet found its way into clinical use, but it is widely applied in scientific studies. Especially for measuring burn depth, assessing wound healing and endothelial function this method is used. Especially in dermatology, plastic surgery, and rheumatology this method is used for investigating microcirculation. However, since no absolute values of red blood cell fluxes are available, a widespread clinical use is not reached yet.

In research settings, **intravital microscopy** serves as a very good microcirculatory monitor. It allows visualization of the interaction of blood components with the endothelium and the leakage of macromolecules into the tissue.<sup>[76]</sup> The vessels are stained with a fluorescent dye. The region of interest is illuminated with light of short wavelengths which excites the dyed molecules. Fluorescent light is emitted which can be seen in the microscope. Since intravital microscopy usually requires the application of toxic fluorescent dyes, its use is limited to animal experiments. Some human applications of intravital microscopy, like **nail fold and skin capillaroscopy** have been developed.<sup>[76, 77]</sup>

In nail fold capillaroscopy, microcirculation under the nails of finger and toes is visualized. The measuring instrumentation is large and expensive and therefore not usable for bed side measurements.<sup>[76]</sup>



Figure 3: Nail fold capillaroscopy. With permission from Distelkamp Electronic<sup>[78]</sup>

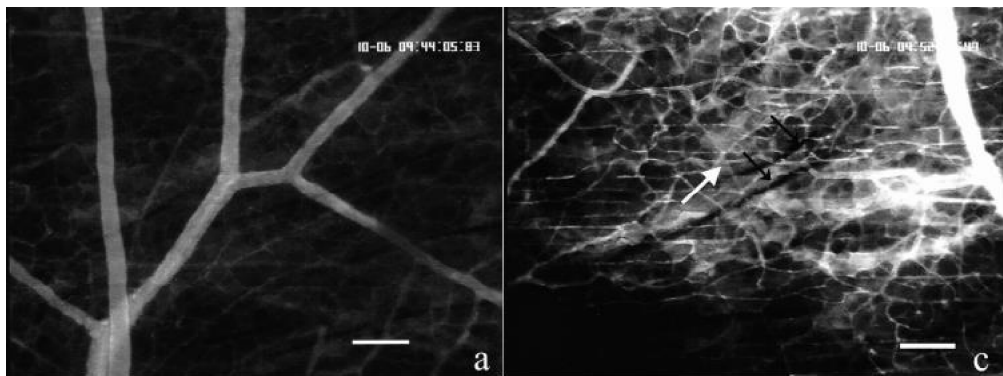


Figure 4: Fluorescence intravital microscopy. With permission<sup>[79]</sup>

Another methodical approach to study microcirculation is **Orthogonal Polarization Spectral (OPS) Imaging**. This microscopy method illuminates the target with linearly polarized light (the light passes a polarizer) and uses a second polarizing filter (analyzer, orientated orthogonally to the polarizer) in front of the camera lens.<sup>[79]</sup> Reflected light preserves the polarization state of the light and this holds also true for single scattering events. However, after multiple scattering events (more than ten scattering events are required) the backscattered light which is remitted from deeper layers of the target

(from depth larger than ten times the single scattering length) is not polarized any more. This depolarized light can be used for imaging microcirculation similar to conventional transmission microscopy. For imaging the microcirculation, a wavelength of 548 nm is used.<sup>[79]</sup> At this wavelength, the oxy- and deoxyhemoglobin have the same absorption coefficient. The blood-filled vessels appear dark on a lighter background. Typically, a field of view of 1 mm<sup>2</sup> is used, depending on the microscopy setting. This optical arrangement is called Mainstream technique, whereas a modification of the system, the **Sidestream Dark Field Imaging (SDF)**, uses light emitting diodes (LED) positioned concentrically around the front lens for illumination. This modification increases the image contrast.<sup>[80]</sup>

Another method which is used is for assessment of microcirculation is **Near-infrared spectroscopy**. This method was used in this thesis and will be discussed in detail in the following section.

## **1.4 Near-infrared spectroscopy**

### **1.4.1 Background**

The first in-vivo near-infrared spectroscopy (NIRS) measurements were done by Frans F. Jöbsis in 1977.<sup>[81, 82]</sup> NIRS was first used for non-invasive investigation of cerebral oxygenation and, later on, for kidney, intestine, and muscle oxygenation in adults. In 1985, NIRS was used to study cerebral oxygenation in sick newborn infants for the first time.<sup>[83]</sup> Since the first clinical application in 1977, many studies have been performed, and NIRS is of increasing interest in various research fields. However, NIRS has not yet been established in routine clinical care of the sick neonate.<sup>[84]</sup>

Near-infrared spectroscopy is a promising technique for the future. Since it is a non-invasive, non-radiative, and painless technique, it is a good method for continuous measuring of the cerebral and peripheral oxygenation in preterm and term neonates. Studies assessing parameters potentially influencing the peripheral oxygenation and perfusion in neonates have been performed.<sup>[85]</sup>

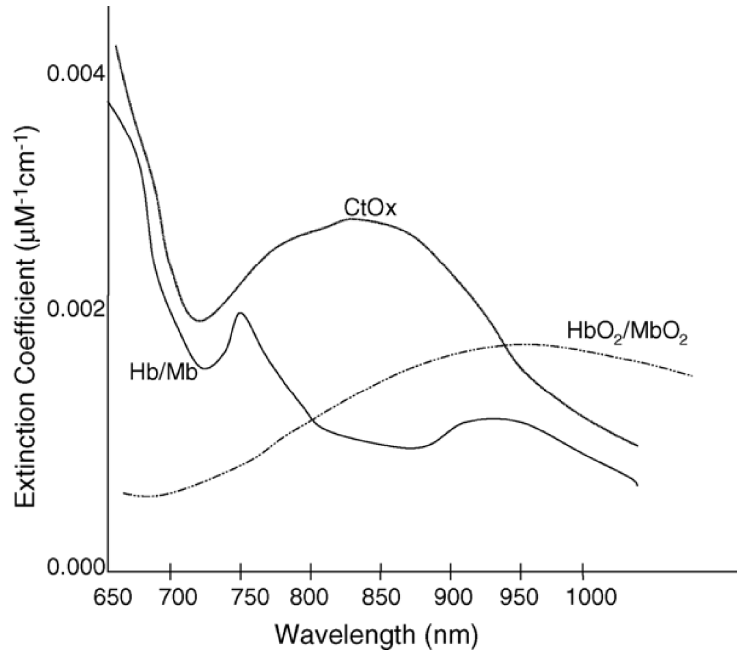
Infections are a common complication in neonates and can quickly lead to death. Studies investigating the effect of sepsis on the microcirculation have been performed.<sup>[86, 87]</sup> A study published in 2011, showed that an elevated CrP level influenced the peripheral tissue oxygenation and perfusion in neonates.<sup>[86]</sup>

### **1.4.2 Measurement principles of near-infrared spectroscopy**

The NIRS method uses the transmission window for near-infrared light in biological tissues for gaining biological information encoded in the back-scattered infrared light.

Visible light with wavelengths of 380 to 700 nm does not penetrate biological tissue more than approximately 1 cm because of absorption and scattering by the tissue components.<sup>[88]</sup> Wavelengths between 700 nm and 3000 nm show less attenuation and therefore a better penetration into biological tissues. Above a wavelength of 900 nm, electromagnetic waves are strongly absorbed by water. Therefore, wavelengths above 900 nm are not used for NIRS. The appropriate range of wavelengths for near-infrared spectroscopy is between 700 and 900 nm.<sup>[89]</sup>

How much light is scattered, depends on the composition of the tissue. Light absorption depends on optical characteristics of the specific molecules. These molecules include chromophores (i.e. chemical groups that form dyes) whose absorption of near-infrared light is oxygen dependent. Oxyhemoglobin ( $\text{HbO}_2$ ), deoxyhemoglobin ( $\text{HHb}$ ), and oxidized cytochrome oxidase ( $\text{CtO}_x$ ) have characteristic and therefore distinguishable absorption spectra in the near-infrared range between 700 and 900 nm.<sup>[84, 88]</sup>



**Figure 5: NIRS absorption spectra for oxyhemoglobin ( $\text{HbO}_2$ ), oxymyoglobin ( $\text{MbO}_2$ ), deoxyhemoglobin ( $\text{HHb}$ ), myoglobin ( $\text{Mb}$ ), and cytochrome oxidase ( $\text{CtO}_x$ ) in equal concentration. The extinction coefficient  $e$  is defined by:  $e = \mu / C$ , with  $\mu$  being the absorption coefficient and  $C$  the concentration. With permission<sup>[90]</sup>**

The basis for the NIRS-Measurement is the Beer-Lambert Law. The modified Beer-Lambert Law is used for measurements of change in oxygenated hemoglobin ( $\Delta\text{HbO}_2$ ), deoxygenated hemoglobin ( $\Delta\text{HHb}$ ), and total hemoglobin ( $\Delta\text{cHb}$ ).

According to the Beer-Lambert law, the radiation intensity  $I(d)$  decreases exponentially with the optical path length  $d$ . The incident light intensity is termed  $I_0$ . The absorption  $A$  depends on the absorption coefficient  $\mu$  of the material and  $\mu$  is equal to the coefficient  $\varepsilon^*$  times the chromophore concentration  $c$ :

$$I(d) = I_0 \cdot e^{-\mu d}$$

with  $\mu = \varepsilon^* \cdot c$  we get

$$I(d) = I_0 \cdot e^{-\varepsilon^* c d}$$

$$\frac{I(d)}{I_0} = e^{-\varepsilon^* c d} \quad / \ln$$

$$- \ln \frac{I(d)}{I_0} = \varepsilon^* c d$$

Per convention, the extinction coefficient  $\varepsilon$  is:

$$\varepsilon = \lg(e) \varepsilon^*, \text{ because: } \lg(x) = \lg(e) \cdot \ln(x); \lg(e) = 0.43429\dots$$

Applying this, we get:

$$- \lg \frac{I(d)}{I_0} = \varepsilon c d$$

According to logarithmic calculation rules:

$$A = \lg \frac{I_0}{I(d)} = \varepsilon c d .$$

However, the law can only be applied when there is no light scattering along the optical path in the tissue. In tissue spectroscopy, photons must transverse different types of tissues and therefore are, to varying degrees, scattered, reflected, or absorbed. Since photons do not travel directly from the source to a receiver, the effective path length is longer than the inter-optode distance. Therefore, the Beer – Lambert law has to be modified<sup>[90]</sup>:

$$A = \sum \varepsilon c d f$$

$d$  is the inter-optode distance and DPF the differential path length factor (in the formula I use  $f$  instead of DPF). The absorption  $A$  is determined by the sum of the absorption contributions of the individual components.  $DPF$  is a multiplication factor (bigger than 1) that calibrates the effective path length  $d_{eff}$  for a given component. It has been reported that  $d_{eff}$  is proportional to  $d$  and the  $DPF$  ( $d_{eff} = d \cdot f$ ), particularly for optical path lengths longer than 3 cm.<sup>[91]</sup> If the DPF is known,  $d_{eff}$  can be calculated. There are typical DPF values reported. In this study, a DPF of 4.2 for was used.

For quantitative measurements, the DPF is necessary to correct the difference between the inter-optode spacing and the effective optical path length  $d_{eff}$ .

The determination of DPF still is one of the major problems for the standardization of NIRS measurements.

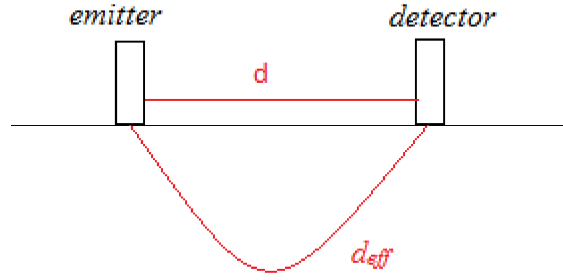


Figure 6: Schematics representing the inter-optode distance  $d$  and the effective optical path length  $d_{eff}$

### 1.4.3 Near-infrared spectroscopy measurement techniques

There are three different NIRS measurement techniques.

#### I. Time resolved near-infrared spectroscopy

Time resolved NIRS measures the time it takes the light pulse to pass through the tissue. For this measurement, a fast detector detects several wavelengths of the picosecond light pulse. With this technique, it is possible to measure absolute chromophore concentrations. Devices using the time resolved NIRS are not commonly used as a bedside monitor, since the devices are very big and expensive.<sup>[84, 92]</sup>

#### II. Phase resolved near-infrared spectroscopy

These devices measure amplitude and phase shift of a light with a known frequency. The absolute oxy- and deoxyhemoglobin concentrations can be measured with this technique. Homogeneity of the tissue is assumed.<sup>[93]</sup>

#### III. Continuous wave near-infrared spectroscopy

Light with different wavelengths is emitted by a laser and is absorbed and scattered by the tissue. An algorithm is used to calculate the concentration changes of the chromophores.<sup>[94]</sup> The calculation of the concentration change is based on the absorption change rate at a certain wavelength, which corresponds to a light intensity change at the photon detector. This method does not consider the exact path length.

Therefore, only relative concentration changes can be studied. Quantitative measurements are not possible.<sup>[84]</sup>

**Continuous wave spatially resolved near-infrared spectroscopy (SRS)** is a subtype of continuous wave technology that allows a quantitative measurement of tissue oxygenation in terms of the tissue oxygenation index (TOI). TOI, which is the ratio of oxygenated hemoglobin to total hemoglobin, represents the vascular oxygen saturation in the compartments penetrated by the light, but it is strongly influenced by the venous compartment.<sup>[95]</sup>

#### 1.4.4 NIRO-200NX measurement principles

In the NIRO-200NX (the device used for the measurements in this project), the emission probe emits three near-infrared light beams of different wavelengths. To measure the change of light attenuation, the NIRO-200NX employs a two-segment photodiode chip in the detection probe.<sup>[91]</sup> The light detected by the photodiodes depends on the distance between the light source and the individual detectors. These measurements are used to estimate the tissue's absorption coefficient times the scattering coefficient. Based on in vivo measurements, an algorithm has been developed which is used in the measurement system to assess the total tissue absorption coefficient. These assessments are performed for each of the wavelengths. Absolute concentrations of HHb, HbO<sub>2</sub>, and cHb are measured.<sup>[91]</sup>

According to the photon diffusion theory, the relative concentrations  $k \cdot HbO_2$  and  $k \cdot HHb$  can be calculated from the measurement of the change of absorption with increasing distance between the optodes. This differential change of absorption with distance is measured by means of the two photo diodes arranged at a given distance in the detection probe. In order to eliminate the constant  $k$  in the equation of the photon diffusion theory, three measurements at three different wavelengths are necessary and

$$\text{TOI results as: } TOI = \frac{k HbO_2}{k HHb + k HbO_2} = \frac{HbO_2}{cHb}. \quad [91, 96]$$



### 1.4.5 Calculation of hemodynamic parameters

Further hemodynamic parameters can be calculated with the help of measuring oxygenated and deoxygenated hemoglobin. These parameters include the tissue oxygenation index (TOI), mixed venous oxygenation (SvO<sub>2</sub>), fractional oxygen extraction (FOE), oxygen delivery (DO<sub>2</sub>), oxygen consumption (VO<sub>2</sub>), and hemoglobin flow (Hbflow/min).<sup>[85]</sup>

With the help of venous occlusion these parameters can be determined. A venous occlusion of a limb is performed, i.e., the venous outflow is occluded, but the arterial inflow is not influenced. This causes an increase in HbO<sub>2</sub>, HHb and cHb. The rate of the increase is a function of the blood flow.

The calculations of the hemodynamic parameters are described below:<sup>[85, 97, 98]</sup>

- **Hemoglobin flow (Hbflow):** hemoglobin flow per minute  
$$\text{Hbflow} = \Delta \text{cHb} / \text{min}$$
- **Oxygen delivery (DO<sub>2</sub>):** amount of oxygen transferred to the tissue  
$$\text{DO}_2 = \text{Hbflow} \cdot 4 \cdot \text{SaO}_2$$
- **Oxygen consumption (VO<sub>2</sub>):** amount of oxygen consumed by the tissue  
$$\text{VO}_2 = \text{Hbflow} \cdot 4 \cdot (\text{SaO}_2 - \text{SvO}_2)$$
- **Mixed venous oxygenation (SvO<sub>2</sub>):** the regional venous oxygenation  
$$\text{SvO}_2 = \frac{\text{HbO}_2}{\text{cHb}}$$
- **Fractional oxygen extraction (FOE):** the proportion of VO<sub>2</sub> to DO<sub>2</sub>  
$$\text{FOE} = \frac{(\text{SaO}_2 - \text{SvO}_2)}{\text{SaO}_2}$$

### 1.4.6 Quality criteria

The main problems of NIRS measurements are the low reproducibility and accuracy. Effort has been made to increase accuracy of NIRS measurements.

Hassan et al. showed that in measurements with arterial occlusion, results for oxygen consumption (VO<sub>2</sub>) are more consistent when accepting only measurements with R<sup>2</sup>>0.96 (assessed with regression analysis).<sup>[99]</sup>

Pichler et al. introduced two quality criteria to increase reproducibility and accuracy.<sup>[100]</sup>

**a. First quality criterion**

During venous occlusion, measurements have to show linear changes in cHb with  $R^2 > 0.95$  (assessed with linear-regression analysis).<sup>[100]</sup>

**b. Second quality criterion**

Pichler et al.:<sup>[100]</sup>

*NIRS measures Hb oxygenation in venules, capillaries, and arterioles. TOI represents the mean Hb oxygenation/saturation of the venous, capillary and arteriolar compartment of a regional tissue. If NIRS is used in combination with venous occlusion, SvO<sub>2</sub> is calculated only from changes in the venous compartment. Therefore*

$$TOI \geq SvO_2 \quad (1)$$

*In peripheral muscle tissue, proportion of venous, capillary, and arteriolar were described as 70:20:10%.<sup>[101]</sup> Assuming that regional tissue oxygen extraction corresponds in venules to OE (SaO<sub>2</sub>-SvO<sub>2</sub>) and that it is between zero and OE (half of OE) in capillaries and not significant in arterioles,<sup>[102]</sup> the “regional tissue” oxygen extraction can be calculated from*

$$(OE \times 0.7) \times 1 + (OE \times 0.2) \times 0.5 + (OE \times 0.1) \times 0 = OE \times 0.8$$

*Thus, the regional tissue oxygen extraction should be 20% lower than OE. However, recent studies using in vivo microelectrode, phosphorescence, or hemoglobin saturation methods have shown that, especially at rest, OE starts in arterioles and continues in capillaries.<sup>[103]</sup> Therefore, regional tissue extraction can be assumed to be even less than 20% lower than OE. TOI, which corresponds to regional tissue hemoglobin oxygenation/saturation including the venous, capillary, and arteriolar compartment, should therefore be up to 20% of OE higher than SvO<sub>2</sub>:*

$$TOI - SvO_2 \leq OE \times 0.2 \quad (2)$$

*Taking into account equation 1 and 2 measurements have to fulfill the following second criteria:*

$$0 \leq TOI - SvO_2 \leq OE \times 0.2$$

=

$$0 \leq TOI-SvO_2 \leq (SaO_2-SvO_2) \times 0.2$$

In this study, we used both the first and the second quality criterion to increase the accuracy of the NIRS measurements.

## 1.5 Echocardiography

Kluckow et al.<sup>[104]</sup> summarize the development of echocardiography in the neonatal intensive care unit in the last years: Echocardiography in neonatology is becoming more and more important as a bedside procedure to evaluate cardiac function and hemodynamics. Pediatric echocardiography performed by pediatric cardiologists provides detailed information of cardiac function, structure and hemodynamics.<sup>[104]</sup> A few years ago, almost every echocardiographic examination was done by a pediatric cardiologist. In the last years, there has been a trend towards neonatologists performing functional echocardiography at the neonatal intensive care units themselves. Since the evaluation of cardiac function and hemodynamics can be performed repeatedly over a longer period of time by a neonatologist, the trend of cardiac function can be assessed more precisely. For assessing changes in hemodynamics shortly after birth, echocardiography is of great value. Only the most important functional and hemodynamic parameters, like intra- and extracardial shunts, heart function, systemic, and pulmonic blood flow are evaluated. It is important to point out that the information obtained by functional echocardiography differs from the information provided by a pediatric cardiologist.

In usual clinical practice, the cardiovascular function is still assessed only by the common methods like clinical signs, continuous heart rate, invasive blood pressure monitoring, and indirect parameters like serum lactate levels and urine output. All these parameters capture the complex cardiac function and its changes after birth only indirectly and therefore have their limitations.<sup>[105]</sup>

Functional echocardiography provides a promising tool for understanding the pathophysiology on an advanced level and can help to guide treatment choices.<sup>[104]</sup>

Echocardiographic parameters for evaluation of the cardiac function include the “tricuspid annular plane systolic excursion” (TAPSE) and the “left ventricular ejection fraction” (LVEF).<sup>[106]</sup>

TAPSE is an echocardiographic measurement to assess the function of the right ventricle. It is a very simple, repeatable, and highly reproducible parameter. Many studies have been performed in adults with and without cardiac defects.<sup>[106]</sup> Recently, studies also on pediatric and neonatal patients showed good and reproducible

results.<sup>[107]</sup> Koestenberger et al.<sup>[107]</sup> could already publish reference values of the TAPSE for term and preterm neonates. LVEF is a parameter assessing the function of the left ventricle.<sup>[107]</sup>

Also of great interest in the neonatal phase is the evaluation of shunts. Gournay et al.<sup>[36]</sup> and Schneider et al.<sup>[43]</sup> have reviewed the ductus arteriosus (DA), which is of special interest in neonates: Echocardiography is the procedure of choice for diagnosis and quantification of a patent ductus arteriosus (PDA). With the help of Color Doppler, the degree of shunting can be evaluated. M-mode echocardiography is used for evaluation of left-ventricular systolic function and for assessment of the ventricle sizes. With these measurements, the magnitude of the shunt can be assessed. Neonates with a small DA usually have normal chamber sizes. In patients with a larger DA, the left side of the heart (atrium and ventricle) is usually enlarged. A left atrium to aorta ratio of smaller than two is said to be a reliable marker of a hemodynamically significant ductal shunt. With the help of Color Doppler, even small DA can be detected. Blood flowing from the aorta to the pulmonary artery (left-to-right shunt) can be seen as a color-coded flow. The ductal flow can be further evaluated to create a pulmonary artery pressure curve. Findings such as right ventricular hypertrophy, septal flattening and pulmonary reflux may suggest the presence of a PDA.

## 2 Materials and Methods

Data for this thesis study (*The influence of a patent ductus arteriosus on the peripheral muscle oxygenation and perfusion in neonates*) were collected as secondary outcome parameters from prospective observational studies which were conducted at the Division for Neonatology at the Department of Pediatrics, Medical University of Graz, Austria.

- I. Der Einfluss von hämodynamischen Parametern auf die zerebrale Oxygenierung bei beatmeten Frühgeborenen mit und ohne arterieller Hypotonie während der ersten Lebenstage. (ethical approval number: 23-402 ex 10/11)
- II. Title: Avoiding hypotension in preterm neonates (AHIP) (ethical approval number: 25-237 ex 12/13)

In addition to the analysis of the existing data set, I added a series of new measurements (from 9 patients) within the framework of the AHIP study.

### 2.1 Patients

Neonates who were admitted to the Neonatal Intensive Care Unit (Division for Neonatology, Medical University of Graz) were included in the study, provided that written parental consent was obtained. Neonates of all gestational ages and both types of delivery (vaginal and caesarean section) were included. A further inclusion criterion was an echocardiography which was done between six hours prior to six hours after the peripheral muscle NIRS measurement.

Exclusion criteria were congenital cardio-pulmonary malformations. Based on the echocardiographic examination, patients were grouped into those with open DA and closed DA.

### 2.2 Study exclusion

All parents were informed that an exclusion of the study was possible at any time without giving any reason for their decision. If this was the case, the neonate was immediately excluded from the study and already collected data was not analyzed.

Furthermore, the parents were ensured that an exclusion of the study would not have any consequences on the further treatment of the newborn.

## **2.3 Demographic and clinical data**

Prior to the measurement, the following data were collected:

- date and time of birth
- type of delivery (vaginal or caesarean section)
- weight, length, and circumference of the head
- APGAR Score
- pH of umbilical arteria and veins
- CrP and Hb on the day of measurement
- medication of the neonate
- diagnosis of the mother
- age at time of NIRS measurement

The diagnosis of the neonate's mother included early uterine contractions, HELLP Syndrome, Gemini pregnancy, in vitro fertilization, placenta praevia, poly-/oligo hydramnion or isthmocervical insufficiency.

One child received Dobutamine during the time of measurement.

## 2.4 Near-infrared spectroscopy measurement

### 2.4.1 NIRO 200-NX

For the NIRS measurements, the NIRO 200-NX (Hamamatsu Photonics, Japan) was used. The NIRO 200-NX is a small device which can be used easily in every intensive care unit as a bedside instrument. Since it has two channels, it is possible to measure simultaneously at two locations (e.g. brain and peripheral muscle).



Figure 7: NIRO 200-NX (Hamamatsu photonics, Japan)<sup>[108]</sup>

With the help of a plastic fixture, the emitter and detector can be held in the right distance from each other; for the cerebral measurement 4 cm, and for the peripheral muscle measurement between 2 and 4 cm. The distance between the emitter and the detector in the peripheral muscle measurement depends on the desired penetration depth and therefore on the diameter of the limb.



Figure 8: Detector (left) and Emitter (right)





Figure 9: Emitter (left) and detector (right) in the plastic fixture (3 cm distance)

The NIRO 200-NX uses LED light with three different wavelengths (735, 810, and 850 nm) and two detectors spaced at 0.8 cm distance to each other.

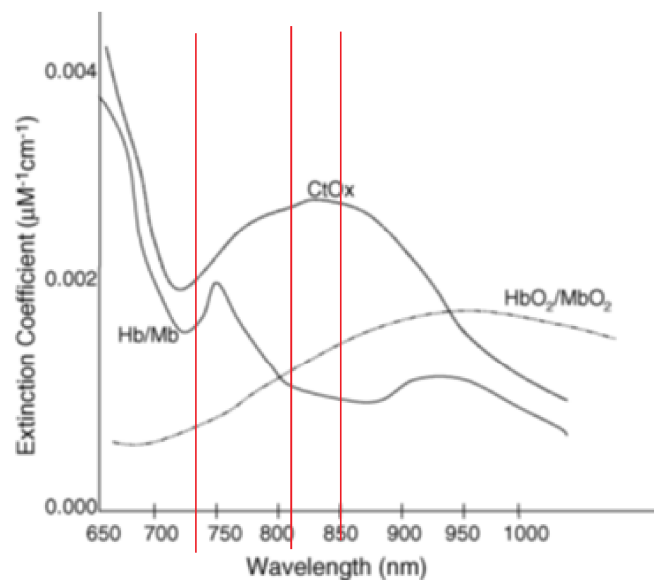


Figure 10: NIRO 200-NX uses three different wavelengths; 735 nm, 810 nm, and 850 nm, marked as red lines (with permission, modified after<sup>[90]</sup>)

The NIRO 200-NX uses both, the modified Beert-Lambert-Law and the spatially resolved spectroscopy (SRS). With the modified Beert-Lambert-Law, it is possible to measure concentration changes of HbO<sub>2</sub>, HHb, cHb, and cytochrome oxidase, whereas with the SRS it is possible to measure the TOI.

#### 2.4.2 Performing the NIRS measurement

*“Measurements were performed under standardized conditions during undisturbed daytime sleep after feeding. The infants laid in a supine position tilted up ( $10^\circ$ ) and the calf was positioned just above the level of mid sternum. Heart rate and arterial oxygen saturation ( $SaO_2$ ) were measured by pulse oximetry on the ipsilateral foot. A skin sensor placed on the ipsilateral calf continuously measured the peripheral temperature. Central and peripheral capillary refill times were assessed with a glass scoop. After positioning of the NIRS optodes, pneumatic cuff, temperature and pulse oximetry sensors, the neonates were left to settle until they had been lying completely still for a minimum of 3 min. Afterwards, arterial blood pressure was measured by an oscillometre with the pneumatic cuff on the thigh.”<sup>[85]</sup>*

For the measurement, the emitting and detecting sensors were placed in the plastic fixture with the corresponding inter-optode distance. The inter-optode distance varied between 2 and 4 cm, depending on the calf diameter.

The cerebral NIRS sensor was placed on the forehead where it was fixed with a fixation bandage. The peripheral NIRS sensor was fixed with a patch at the lower leg above the Musculus gastrocnemius. The sensors were fixed with as little pressure as possible and without circular fixation to avoid the pressure to be higher than venous pressure.



**Figure 11: Central NIRS measurement**



**Figure 12: Peripheral NIRS measurement setting - blood pressure cuff, NIRS sensors, and pulse oximeter at the same leg**

Venous occlusions were performed by inflating the cuff to a pressure between venous and diastolic arterial pressure (20-30 mmHg). *“The cuff was maintained inflated for 20 seconds and NIRS data were recorded. Changes in HbO<sub>2</sub>, HHb, and cHb during venous occlusion were caused only by arterial inflow and oxygen consumption of the tissue.”*<sup>[85]</sup> A linear increase of HbO<sub>2</sub>, HHb, and cHb during the occlusion could be seen on the NIRS monitor. If the neonate started to move the measurement was interrupted and repeated after another resting period of at least one minute.



**Figure 13: Linear increase of  $\Delta cHb$ ,  $\Delta HbO_2$ ,  $\Delta HHb$  during venous occlusion**

Measurements were repeated until at least one measurement passed the first quality criterion published by Pichler et al.<sup>[100]</sup> (compare to chapter 1.4.6). Concentration changes of the following parameters were measured during venous occlusion:

- Oxygenated hemoglobin ( $HbO_2$ )
- Deoxygenated hemoglobin ( $HHb$ )
- Total hemoglobin ( $cHb$ )
- Tissue oxygenation index ( $TOI$ )

From the obtained measurements of  $HbO_2$ ,  $HHb$ ,  $cHb$ , and  $TOI$  further parameters were calculated:  $Hbflow$ ,  $SvO_2$ ,  $DO_2$ ,  $VO_2$ , and  $FOE$ . For calculation and definition of details compare to chapter 1.4.5.

## 2.5 Echocardiography

Echocardiography was performed within a time frame of  $\pm 6$  hours from NIRS measurements. For echocardiography, the Logiq S8 (GE Healthcare GmbH Solingen, Germany) was used. Echocardiographic measurements included identification of structural heart diseases and assessment of the DA. The diameter was then related to the body weight of the neonate.

The echocardiography included:

- The identification of structural heart diseases
- The assessment of the ductus arteriosus
  - In a high left parasternal window using pulsed Doppler echocardiography and color flow mapping, the diameter of the DA and the direction of flow over the DA were assessed.
  - The diameter was then related to the body weight of the neonate. The DA diameter to body weight ratio was used for further analysis, as there is a significant correlation between early DA diameter and the development of patent DA symptoms.<sup>[109]</sup>

## 2.6 Statistical analysis

Statistical analysis was performed using Microsoft Excel 2010 and SPSS Statistics Version 22.

NIRS measurement data was transferred to a computer, anonymized, and saved in an Excel database. The measurements were checked for the second quality criterion and, depending on the result, included for further analysis or dismissed.<sup>[100]</sup>

Depending on the data distribution, values are given as median and minimum and maximum [min,max] (for not normally distributed data) or mean  $\pm$  SD (standard deviation) for normally distributed data. Testing for normal distribution was done with the Shapiro-Wilk test.

Demographic data and NIRS parameters of preterm neonates with open and closed DA were compared. Depending on the distribution of data, intergroup comparison was performed with Mann-Whitney-U test for nonparametric analysis or with t-test for normally distributed data. P-values of less than 0.05 were considered as statistically significant. Correlation analyses between DA diameter and NIRS parameters were performed. For correlation analysis, Pearson's correlation coefficient and Spearman's rank correlation coefficient were used.

### 3 Results

A total of 40 neonates were included in the study. There were twelve term- and 28 preterm neonates. Their mean gestational age was 35.0 weeks of gestation.

For the statistical analysis, the total of 40 neonates was stratified into 9 further groups:

1. All
2. All with PDA
3. All without PDA
4. Preterm
5. Preterm with PDA
6. Preterm without PDA
7. Term
8. Term with PDA
9. Term without PDA

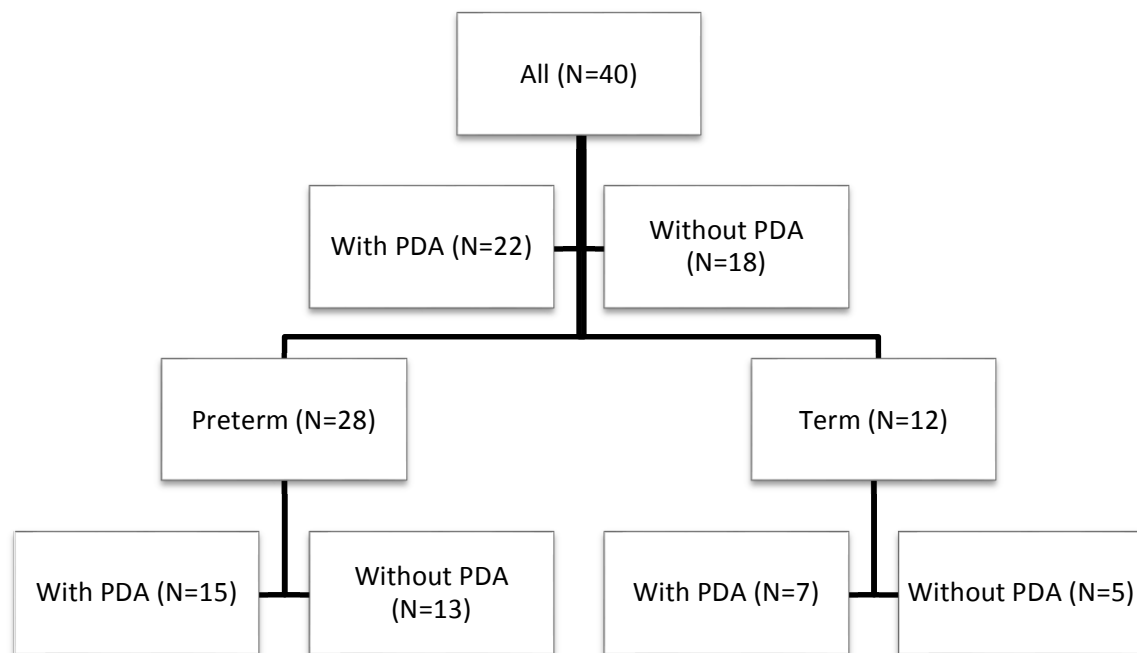


Figure 14: Flow chart of the classification of groups

The measurement was conducted between the first and third day after birth. The mean age at the moment of measurement was 12.9 hours with the earliest measurement 45 minutes after birth and the latest 72 hours after birth.

Six neonates had an elevated CrP on the second day of life, all other neonates had normal CrP values. One neonate received a Dobutamine for support of cardiac function. Two children received Indomethacin, and one child Ibuprofen for closure of the PDA.

The collected data are divided into demographic, clinical, echocardiographic, pulse oximeter, and NIRS parameters.

If data was normally distributed results are expressed as mean  $\pm$  SD. If the data were not normally distributed they are expressed as median [minimum, maximum].



### 3.1 Demographic and clinical data of patients

	All	Term	Preterm	All DA open	All DA closed
Number of newborn	40	12	28	22	18
Sex	26	7	19	17	9
	14	5	9	5	9
Female					
Gestational age [weeks]	34.95 [30.9,41.9]	<b>40.23 ± 1.3*</b>	<b>33.76 ± 1.42*</b>	35.3 ± 3.5	36.2 ± 3.04
Birth weight [g]	2577.575 ± 825.43	<b>3612.5 ± 393.6*</b>	<b>2134.04 ± 490.9*</b>	2240 [1290,4300]	2550 [1380,3850]
Umbilical artery pH (art.)	7.275 [7.1,7.36]	<b>7.2 ± 0.07*</b>	<b>7.3 [7.18,7.36]*</b>	7.27 ± 0.07	7.27 [7.12,7.32]
Umbilical vein pH (ven.)	7.34 ± 0.06	<b>7.3 ± 0.05*</b>	<b>7.4 ± 0.06*</b>	7.34 [7.19,7.51]	7.35 [7.3,7.4]
APGAR 1	8.0 [1.0,9.0]	8.0 [1.0,9.0]	8.0 [5.0,9.0]	8.0 [1.0,9.0]	8.0 [7.0,9.0]
APGAR 5	9.0 [6.0,10.0]	9.0 [6.0,10.0]	9.5 [6.0,10.0]	9.0 [6.0,10.0]	10.0 [8.0,10.0]
APGAR 10	10.0 [8.0,10.0]	9.5 [8.0,10.0]	10.0 [9.0,10.0]	9.0 [8.0,10.0]	10.0 [9.0,10.0]
Ca. Ref. Time sternal [s]	3.14 ± 0.68	3.38 ± 0.89	3.04 ± 0.53	3.32 ± 0.5	3.09 ± 0.77
Ca. Ref. Time peripheral [s]	3.03 ± 0.73	3.36 ± 0.9	2.91 ± 0.58	2.99 [2.07,5.01]	3.02 ± 0.57
BP sys [mmHg]	63.36 ± 9.33	<b>69.42 ± 9.88*</b>	<b>60.56 ± 7.75*</b>	63.95 ± 11.42	62.53 ± 5.55
BP dia [mmHg]	29.5 ± 5.98	31.96 ± 8.25	28.37 ± 4.34	28.82 ± 5.66	30.44 ± 6.48
MAP [mmHg]	41.68 ± 5.83	<b>45.42 ± 6.89*</b>	<b>39.96 ± 4.43*</b>	41.32 ± 6.81	42.19 ± 4.29
Subcutaneous adipose tissue thickness [mm]	0.29 ± 0.1	0.34 ± 0.15	0.26 ± 0.07	0.3 ± 0.1	0.28 ± 0.12

**Table 2: Demographic and clinical data of five groups: all, term, preterm, all with PDA, and all without PDA, \*statistically significant  $p < 0.05$**

	Preterm DA open		Preterm DA closed		Term DA open		Term DA closed	
Number of newborn	15	13			7	5		
Sex								
Male	11	8			6	1		
Female	4	5			1	4		
Gestational age [weeks]	<b>33.1 [30.9,35.6]*</b>	<b>35 [31.6,35.8]*</b>			40.1 [38.1,41.9]	40.4 [40.1,41.7]		
Birth weight [g]	2020 [1290,2820]	2250 [1380,3070]			3710 [3080,4300]	3420 [2980,3850]		
Umbilical artery pH (art.)	7.29 ± 0.05	7.28 [7.18,7.32]			7.2 [7.1,7.34]	7.16 [7.12,7.29]		
Umbilical vein pH (ven.)	7.36 [7.19,7.51]	7.35 [7.3,7.4]			7.28 [7.23,7.37]	Only 2 values		
APGAR 1	8.0 [5.0,9.0]	8.0 [7.0,9.0]			6.0 ± 2.7	9.0 [8.0,9.0]		
APGAR 5	9.0 [6.0,10.0]	10.0 [8.0,10.0]			8.1 ± 1.35	10.0 [8.0,10.0]		
APGAR 10	10.0 [9.0,10.0]	10.0 [9.0,10.0]			9.0 ± 0.9	10.0 [9.0,10.0]		
Cap. Ref. Time sternal [s]	3.16 ± 0.38	3.0 ± 0.75			3.44 ± 1.0	3.29 ± 0.85		
Cap. Ref. Time peripheral [s]	2.81 [2.07,4.66]	2.95 ± 0.62			3.5 ± 1.18	3.19 ± 0.44		
BP sys [mmHg]	60.03 ± 9.37	61.27 ± 5.12			72.36 ± 11.43	65.3 ± 6.03		
BP dia [mmHg]	27.87 ± 4.67	29.05 ± 3.95			30.86 ± 7.34	33.5 ± 10.06		
MAP [mmHg]	39.17 ± 4.99	41.05 ± 3.47			45.93 ± 8.24	44.7 ± 5.25		
Subcutaneous adipose tissue thickness [mm]	0.28 ± 0.06	0.25 ± 0.07			0.30 ± 0.17	0.39 ± 0.17		

**Table 3: Demographic and clinical data of four groups: preterm with PDA, preterm without PDA, term with PDA, and term without PDA, \*statistically significant  $p < 0.05$**

The mean gestational age and the mean birth weight differed significantly between preterm and term neonates. With a value of  $7.2 \pm 0.07$  the mean umbilical artery pH in term neonates was significantly lower than the value of 7.3 [7.18,7.36] in preterm neonates ( $p = 0.005$ ). The mean systolic blood pressure of term neonates was significantly higher than in preterm neonates ( $69.42 \pm 9.88$  mmHg versus  $60.56 \pm 7.75$  mmHg;  $p=0.012$ ). Also, the mean arterial pressure with a value of  $45.42 \pm 6.89$  mmHg was significantly higher in term neonates than in preterm neonates ( $39.96 \pm 4.43$  mmHg) ( $p = 0.006$ ).

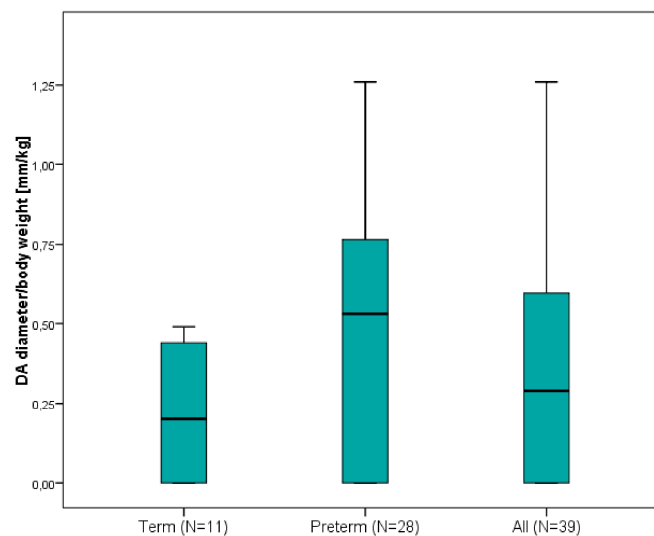
In the group of preterm neonates with open DA, the median gestational age was significantly lower than in the group of preterm with closed DA (33.1 [30.9,35.6] versus 35.0 [31.6,35.8] weeks of gestation;  $p = 0.011$ ).

All other values showed no statistically significant difference.

## 3.2 Echocardiographic results

Within six hours before or after the NIRS measurement, a functional echocardiography was performed. For all groups, the ductus arteriosus diameter was measured and the per kilogram bodyweight diameter was calculated.

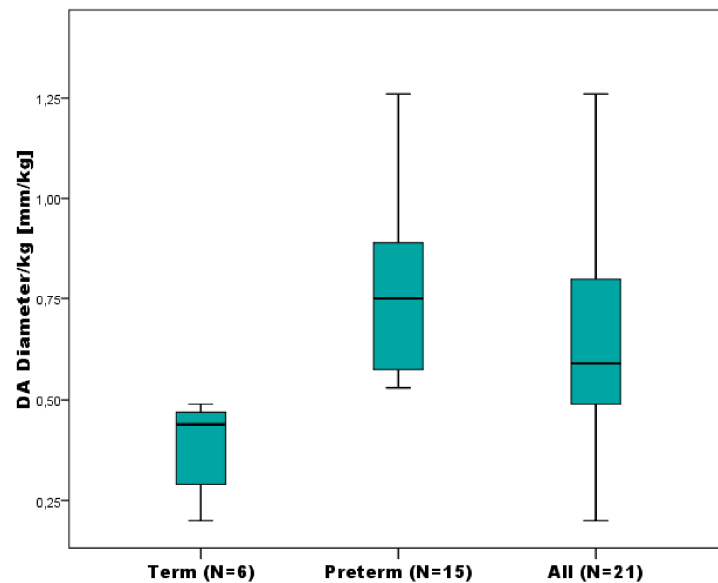
The following boxplots show the DA diameter/body weight for the different groups of term, preterm and all neonates. All data are included in this figure (also closed DA; a closed DA equals a diameter of 0.0 mm/kg).



**Figure 15: DA diameter/body weight for term-, preterm-, and all neonates. All data are included (0 equals a closed DA)**

The median DA diameter/kg of term neonates was 0.2 [0.0,0.49] mm/kg, of the preterm neonates 0.53 [0.0,1.26] mm/kg and of all neonates 0.29 [0.0,1.26] mm/kg. There was no statistically significant difference between term and preterm neonates.

The following boxplots show the DA Diameter/body weight for the different groups of term, preterm and all neonates. Only neonates with an open DA are included in this figure.



**Figure 16: DA diameter/body weight for term-, preterm-, and all neonates. Only neonates with an open DA are included**

The mean value for the term group was  $0.39 \pm 0.12$  mm/kg, the median for the preterm neonates 0.75 [0.53,1.26] mm/kg and the mean diameter for all neonates was  $0.67 \pm 0.29$  mm/kg.

The mean DA diameter/kg for term and preterm neonates differed significantly between the groups ( $p < 0.001$ ).

### 3.3 NIRS parameters and pulse oximeter parameters SpO<sub>2</sub> and HR

	All	Term	Preterm	All DA open	All DA closed
<b>Pulse oximeter</b>					
SaO <sub>2</sub> [%]	97,0 [84.1,100]	98.5 [84.1,100]	96.65 [85.5,100]	<b>95.0 [84.1,100]*</b>	<b>97.33 ± 2.45*</b>
HR [bpm]	129,3 [105.9,185.8]	125.45 [105.9,185.8]	134.8 ± 16.03	<b>139.38 ± 19.87*</b>	<b>126.63 ± 12.89*</b>
<b>NIRS</b>					
pTOI [%]	69.0 ± 5.6	70.24 ± 4.8	68.4 ± 5.87	67.79 ± 5.79	70.55 [54.9,75.7]
DO <sub>2</sub> [μmol l <sup>-1</sup> min <sup>-1</sup> ]	36.5 ± 14.7	34.0 ± 6.77	37.7 ± 17.05	34.89 ± 14.91	38.49 ± 14.56
VO <sub>2</sub> [μmol l <sup>-1</sup> min <sup>-1</sup> ]	10.5 [6.4,18.6]	9.8 [7.4,16.1]	10.6 [6.4,18.6]	10.89 ± 3.74	11.37 ± 3.53
SvO [%]	0.7 [0.5,0.8]	0.7 [0.5,0.8]	0.7 [0.5,0.7]	0.6 [0.5,0.8]	0.7 [0.5,0.7]
FOE	0.3 [0.2,0.5]	0.3 [0.2,0.5]	0.3 [0.2,0.5]	0.3 [0.2,0.5]	0.3 [0.2,0.5]
cTOI [%]	71.38 ± 8.2	67.76 ± 8.1	73.31 ± 7.8	70.55 ± 8.03	72.02 ± 8.58

Table 4: SaO<sub>2</sub>, HR and NIRS parameters of the different groups

	Preterm DA open	Preterm DA closed	Term DA open	Term DA closed
<b>Pulse oximeter</b>				
SaO <sub>2</sub> [%]	<b>94.37 ± 3.62*</b>	<b>96.98 ± 2.5*</b>	95.49 ± 5.81	99.24 ± 2.3
HR [bpm]	140.44 ± 16.35	128.8 ± 13.81	142.98 ± 30.85	120.98 ± 8.9
<b>NIRS</b>				
pTOI [%]	67.3 ± 6.25	70.0 [54.9,75.6]	70.4 ± 5.85	71.7 ± 3.24
DO <sub>2</sub> [μmol l <sup>-1</sup> min <sup>-1</sup> ]	33.05 [15.3,62.1]	41.93 ± 16.27	34.11 ± 7.9	33.74 ± 5.7
VO <sub>2</sub> [μmol l <sup>-1</sup> min <sup>-1</sup> ]	11.03 ± 4.39	12.25 ± 3.85	10.1 ± 2.77	10.3 [7.4,12.2]
SvO <sub>2</sub> [%]	0.6 [0.5,0.7]	0.7 [0.5,0.7]	0.66 ± 0.11	0.7 [0.6,0.7]
FOE	<b>0.35 [0.3,0.5]*</b>	<b>0.3 [0.2,0.5]*</b>	0.31 ± 0.1	0.3 [0.3,0.3]
cTOI [%]	73.9 ± 3.65	67.07 ± 1.63	66.2 ± 3.68	69.33 ± 11.59

Table 5: SaO<sub>2</sub>, HR and NIRS parameters of the different groups

There are statistically significant differences in the mean values of SaO<sub>2</sub> and HR when comparing the two groups of all neonates with DA and all neonates with closed DA. When comparing all neonates with an open DA to the group of neonates with closed DA, a significantly lower SaO<sub>2</sub> was found in neonates with an open DA (95.0% compared to 97.3%,  $p = 0.032$ ).

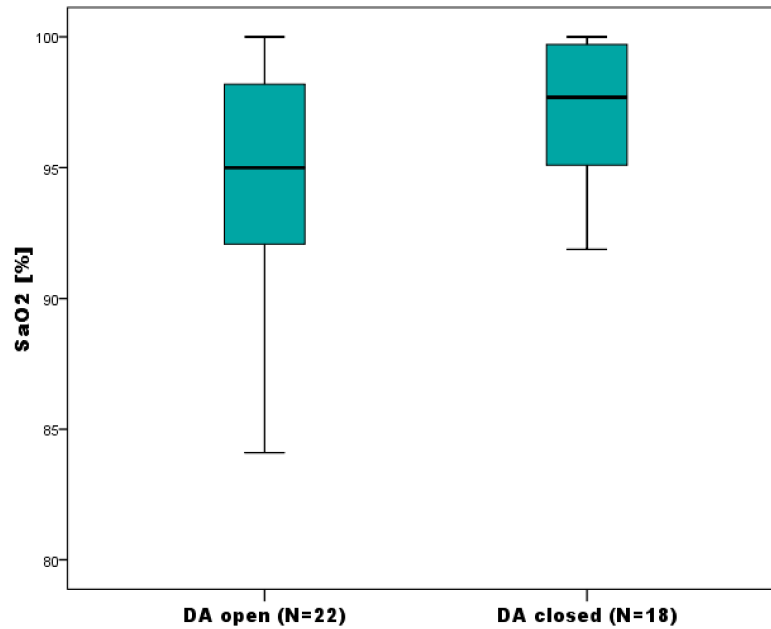


Figure 17: Comparison of SaO<sub>2</sub> values of all neonates with open DA and all neonates with closed DA

The HR was significantly higher in the group of all neonates with an open DA ( $139.38 \pm 19.87$ ) compared to those with a closed DA ( $126.63 \pm 12.89$ ) ( $p=0.022$ ).

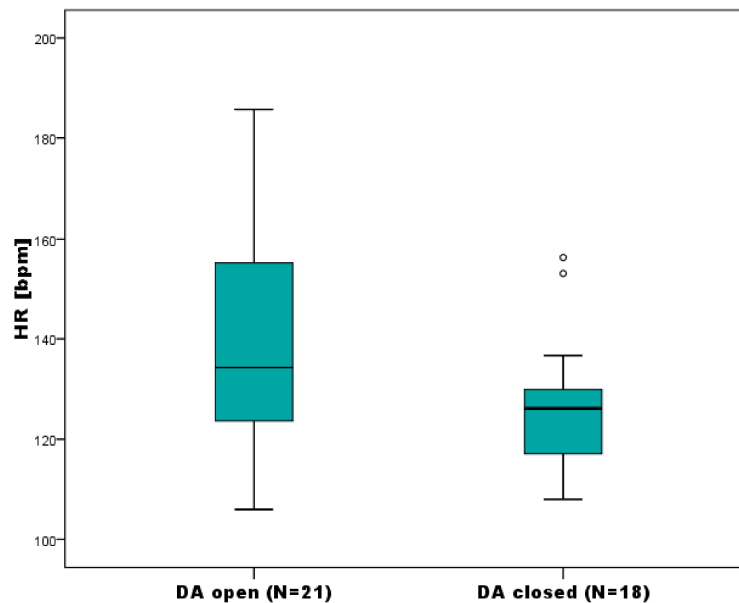


Figure 18: Comparison of HR values of all neonates with open DA and all neonates with closed DA



Also in preterm neonates, the SaO<sub>2</sub> differed significantly in neonates with an open DA compared to those with a closed DA. The values for the preterm neonates with an open DA ( $94.37 \pm 3.62$ ) were significantly lower than those in neonates with a closed DA ( $96.98 \pm 2.5$ ) ( $p = 0.038$ ).

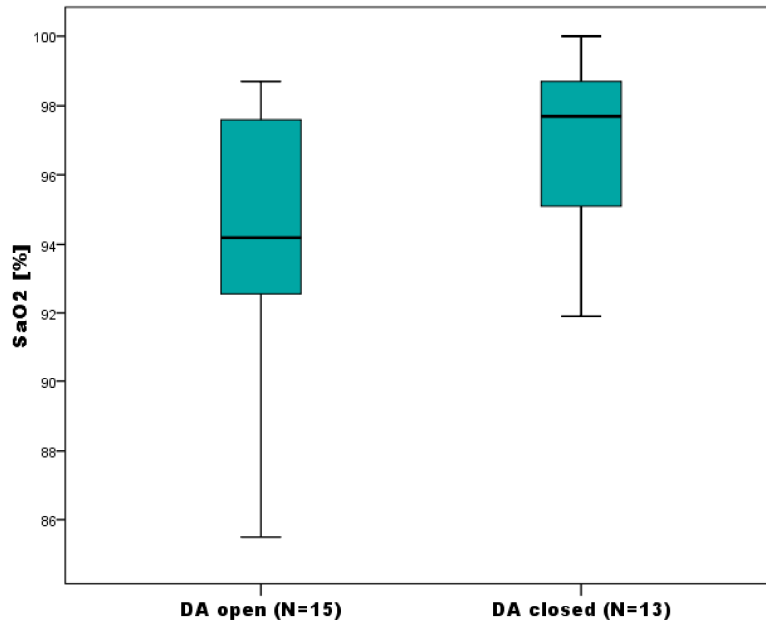


Figure 19: Comparison of SaO<sub>2</sub> values in preterm neonates with open and closed DA

The FOE was higher in preterm neonates with an open DA than in those with a closed DA ( $p = 0.046$ ).

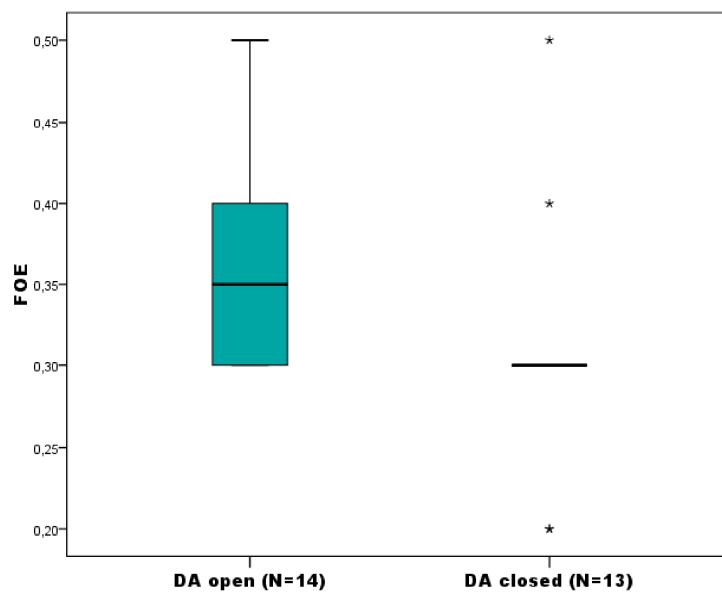


Figure 20: Comparison of FOE in preterm neonates with open DA and closed DA

All other values didn't show any significant differences.

### 3.4 Analysis of correlations between NIRS parameters and ductus arteriosus diameter

For not normally distributed data the Pearson's correlation coefficient and for not normally distributed data the Spearman correlation coefficient was used.

The following table shows the correlations between the NIRS parameters, the pulse oximeter parameters SaO<sub>2</sub> and HR, and the ductus arteriosus diameter/kg.

	All (N =40)	Term (N=12)	Preterm (N=28)
DA diameter/body weight – SaO <sub>2</sub>	<b>r = -0.397 (p=0.012)*</b>	r = -0.081 (p=0.812)	<b>r = -0.377 (p=0.048)*</b>
DA diameter/body weight – HR	<b>r = 0.460 (p=0.004)*</b>	r = 0.477 (p=0.138)	<b>r = 0.489 (p=0.010)*</b>
DA diameter/body weight – pTOI	<b>r = -0.359 (p=0.025)*</b>	r = -0.377 (p=0.252)	r = -0.295 (p=0.127)
DA diameter/body weight – DO <sub>2</sub>	r = -0.162 (p=0.330)	r = -0.334 (p=0.316)	r = -0.172 (p=0.391)
DA diameter/body weight – VO <sub>2</sub>	r = -0.064 (p=0.703)	r = -0.105 (p=0.759)	r = -0.116 (p=0.564)
DA diameter/body weight – SvO <sub>2</sub>	<b>r = -0.394 (p=0.014)*</b>	r = -0.331 (p=0.320)	<b>r = -0.413 (p=0.032)*</b>
DA diameter/body weight – FOE	<b>r = 0.412 (p=0.010)*</b>	r = 0.376 (p=0.255)	<b>r = 0.417 (p=0.030)*</b>
DA diameter/body weight – cTOI	r = 0.054 (p=0.816)	r = -0.638 (p=0.173)	r = 0.226 (p=0.419)

Table 6: Correlations between pulse oximeter parameters, NIRS parameters and DA diameter/body weight (\* statistically significant, p<0.05)

There was a significant negative correlation between DA diameter/kg and SaO<sub>2</sub> in the group of all neonates ( $p = 0.012$ ). We also found this correlation in the preterm group ( $p = 0.048$ ).

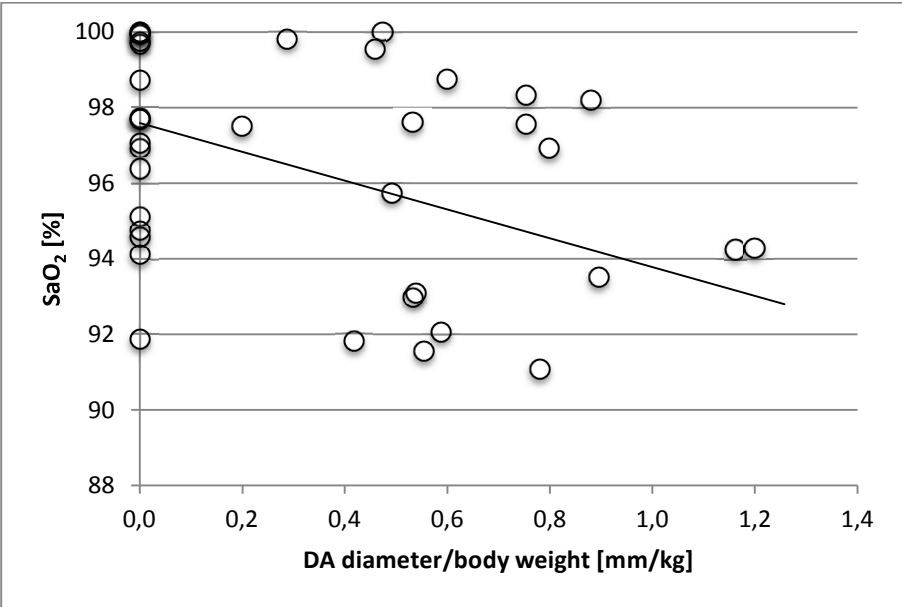


Figure 21: Correlation between DA diameter/body weight and SaO<sub>2</sub> in the group of all neonates

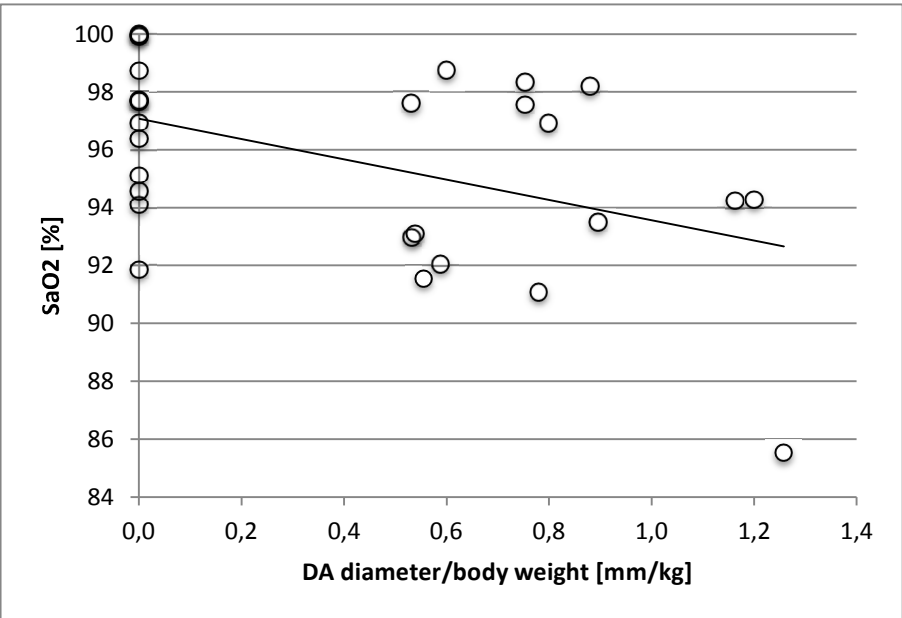


Figure 22: Correlation between DA diameter/body weight and SaO<sub>2</sub> in preterm neonates

Within all neonates, a significant positive correlation was found between DA diameter/body weight and the HR ( $p = 0.004$ ). This correlation was also found in preterm neonates ( $p = 0.010$ ).

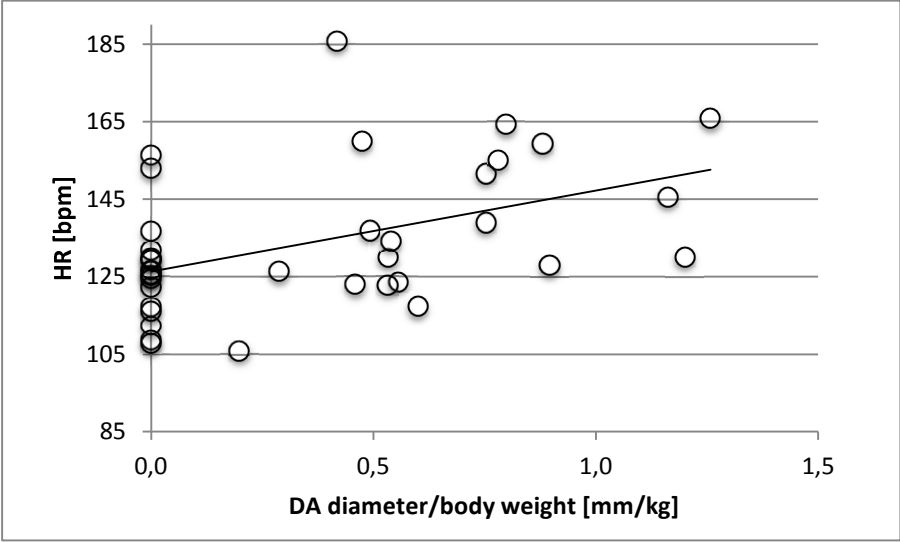


Figure 23: Correlation between DA diameter/kg and HR in the group of all neonates

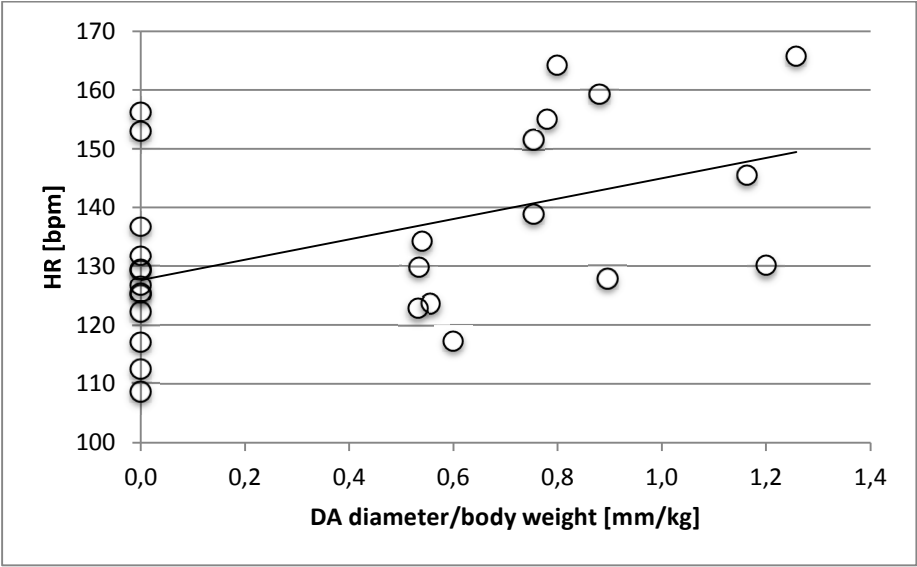


Figure 24: Correlation between DA diameter/body weight and HR in preterm neonates

There was a significant negative correlation between DA diameter/body weight and pTOI in the group of all neonates ( $p = 0.025$ ).

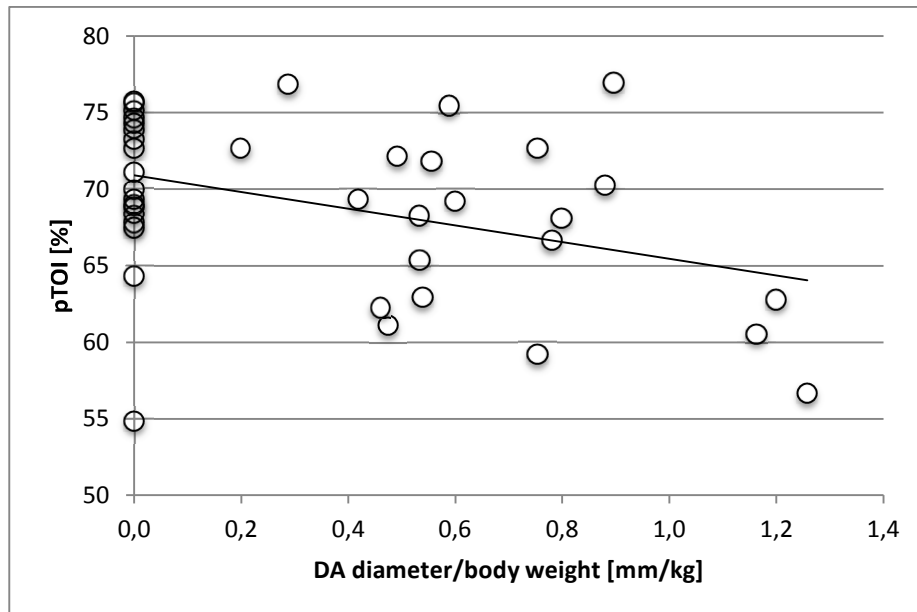


Figure 25: Correlation between DA diameter/kg and pTOI in all neonates

SvO<sub>2</sub> and DA diameter/body weight had a significant negative correlation in the group all 40 neonates ( $p=0.014$ ), as well as in preterm neonates ( $p=0.032$ ).

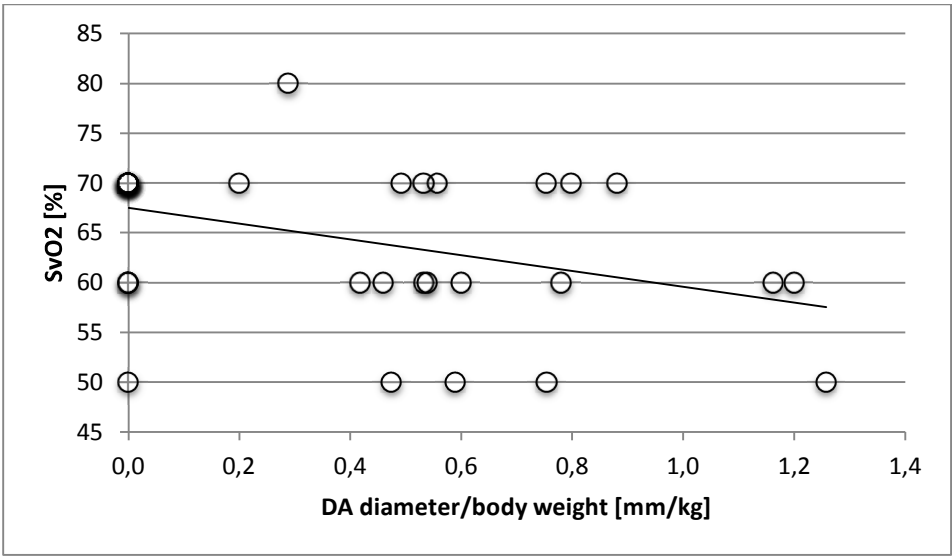


Figure 26: Correlation between DA diameter/body weight and SvO<sub>2</sub> in the group of all neonates

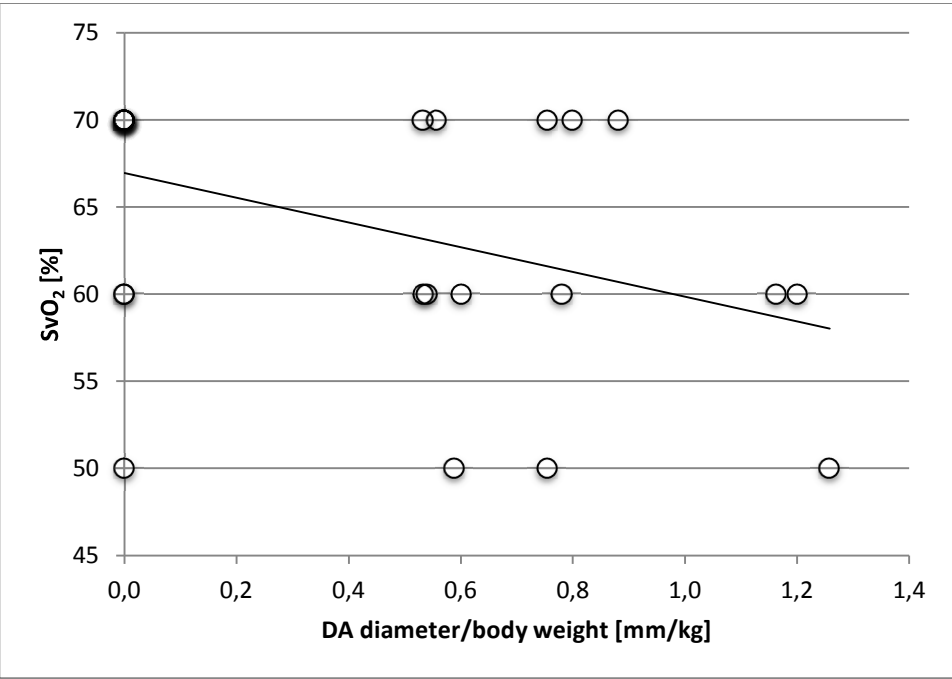


Figure 27: Correlation between DA diameter/body weight and SvO<sub>2</sub> in preterm neonates

In the group of all 40 neonates ( $p = 0.010$ ), as well as in the preterm group ( $p = 0.030$ ), we found a positive correlation between DA diameter/body weight and FOE.

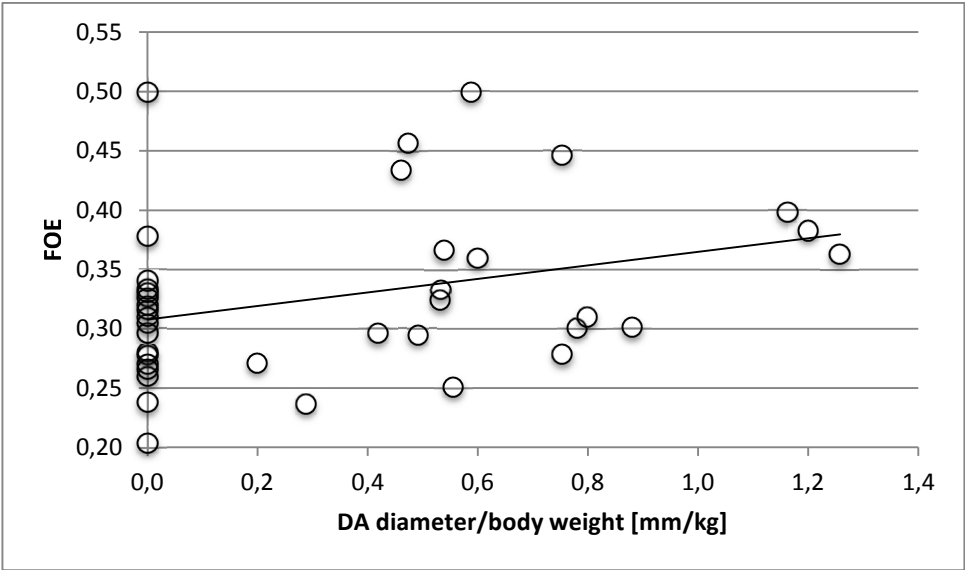


Figure 28: Correlation between DA diameter/body weight and FOE in the group of all neonates

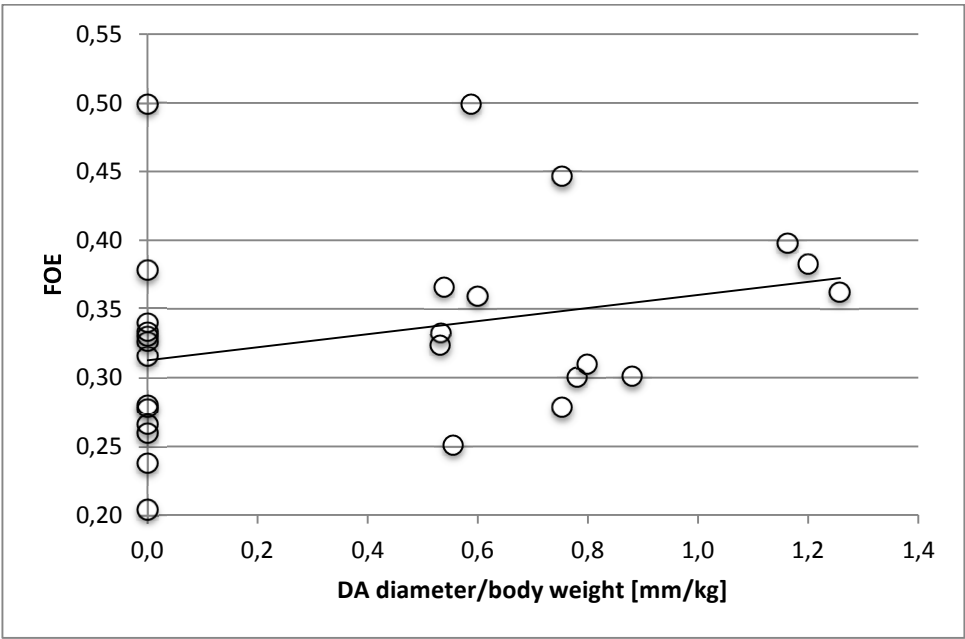


Figure 29: Correlation between DA diameter/kg and FOE in preterm neonates

All other correlations were statistically not significant.

## 4 Discussion

### 4.1 Discussion of the study design

Data for this observational study were collected as secondary outcome parameters in prospective observational studies, which were conducted at the Division for Neonatology at the Department of Pediatrics, Medical University of Graz, Austria.

For this retrospective study using data collected from prospective studies conducted at the neonatal ward from 2010 – 2015, the databases were searched for neonates who received an echocardiography within six hours before or after the near-infrared spectroscopy measurement.

Study limitations:

- The present study represents a post-hoc analysis of already finished studies. The concept of these studies was not planned for the questions of this doctoral thesis.
- There were three different people performing the echocardiography, which may have weakened the results as different observer may decrease reliability.
- Despite the long interval of data collection, only 40 neonates could be included in the study. The limiting factor was that only few children received an echocardiography in the given time frame.



## 4.2 Discussion of the methods used

### 4.2.1 Near-infrared spectroscopy

For this study, the near-infrared spectroscopy (NIRS) method was used. In 1985 NIRS was first applied in neonates and since then various studies have been performed to analyze the NIRS method in neonates.

In recent years, particularly the aspect of specificity and reproducibility of peripheral NIRS measurements have been analyzed. Pichler et al. published a paper in 2008 with recommendations on how to increase the comparability and validity of peripheral NIRS measurements.<sup>[110]</sup> One important aspect they discuss is that measurements should be made when the neonate is at rest. Only then, the measurements will allow comparable and reproducible results. They found that after movement, the resting period should be at least 2 minutes for the blood flow to return to pre-movement levels.<sup>[110]</sup> Sometimes, this proved to be a great challenge while performing the NIRS measurements; some neonates had to be excluded because of restlessness.

Apart from methodical difficulties, there are still some technological problems concerning NIRS. The differential path length factor (DPF) still is one of the major problems. Even though accurate estimates of DPF for different tissues were derived, the path length itself is influenced by age, hemodynamic changes, attachment method, and pressure.<sup>[84, 111]</sup> Depending on the inter-optode distance a fixed DPF was used in our study. The estimation of the DPF is a potential cause of error in our measurements.

Beekvelt et al. found that subcutaneous adipose tissue thickness (ATT) has a substantial confounding influence on NIRS measurements.<sup>[112]</sup> Estimates suggest that the maximum measurement depth in a tissue is half the inter-optode distance. It is therefore essential to measure the exact ATT and to choose the right inter-optode distance for the individual patient. Beekvelt et al. found a major decrease in muscular oxygen consumption with increasing ATT.<sup>[112]</sup> Because the metabolism in fatty tissue is far less than in muscle tissue, it seems likely that measurements were simply performed in the “wrong” tissue. It is therefore essential to capture an ultrasound image for measuring the ATT to choose the right inter-optode distance, particularly if the study populations have a wide range in body weight. We captured a standard ultrasound image for measuring the ATT in every neonate included in the study. A recently developed ultrasound method to quantify subcutaneous adipose tissue (SAT) thickness could be adapted to

quantify the neonate's SAT on a higher accuracy and reliability level and to study this question systematically.<sup>[113, 114]</sup>

The validity of any monitoring instrument depends on its precision and accuracy.<sup>[115]</sup> Several studies have been performed to assess comparability and reproducibility of measurements obtained by two different NIRS systems.<sup>[116, 117]</sup> Pocivalnik et al. compared the INVOS (Somanetics, Troy, MI) and NIRO (Hamamatsu Photonics, Hamamatsu, Japan) instruments with each other. They found a 10% difference in cerebral oxygenation between these two monitors, with NIRO values being lower.<sup>[117]</sup> Sorensen and Greisen studied the precision of TOI using the NIRO 300 monitor (Hamamatsu Photonics, Hamamatsu, Japan). A large intra- and interpatient variability was shown.<sup>[116]</sup> The reasons for these variations are complex and multifactorial. Different manufacturers use different algorithms to calculate the saturation value and there is no calibration standard.<sup>[115]</sup> For our study we used the NIRO-200NX (Hamamatsu Photonics, Hamamatsu, Japan) only.

Furthermore, Dix et al. showed significant differences in the measurement results when sensors for adults and for neonates were used.<sup>[118]</sup> The reason could be related to different calibrations.<sup>[115, 118]</sup>

Pichler et al. introduced quality criteria to increase the reproducibility in NIRS measurements.<sup>[100]</sup> With the introduction of two quality criteria, to increase the reproducibility of peripheral-muscle NIRS measurements and decrease the test-retest variability of TOI, SvO<sub>2</sub>, FOE, Hbflow, DO<sub>2</sub>, and VO<sub>2</sub> measurements.<sup>[100]</sup> We have used these two quality criteria in our measurements, too.

Most of the studies mentioned, examined the instruments when measuring cerebral oxygenation. Because the technique and the uncertainties remain similar when measuring peripheral muscular oxygenation, it can be assumed that the large intra- and interpatient variability remains there, too.

A recently published review by Kenosi et al., points out that NIRS is recently undergoing a great progress since many multicenter and multinational studies are conducted.<sup>[119]</sup> *“With advances in technology and as the evidence base for its use continues to evolve, we may finally have concrete evidence for its use in neonatal care.”*<sup>[119]</sup>

#### **4.2.2 Echocardiography**

Echocardiographic imaging depends largely on the investigator. Since the data was collected over a long period of time, it was not possible to always have the same person performing the echocardiography. The data used in this study were measured by three different neonatal doctors, which may have negative impact on reliability.

The accuracy of quantitative echocardiographic studies is limited by the wavelength of the ultrasound system, the image quality of a given system, and the appropriate parameter setting of the ultrasound instrument. Additional problems of quantitative analyses arise because of heart movement.

The echocardiography was performed in the time span 6 hours before until 6 hours after the NIRS measurement. Therefore, it is possible that slight changes in the PDA diameter occurred during this period of time.

## 4.3 Discussion of results

### 4.3.1 Comparison of PDA diameter per kilogram bodyweight in term and preterm neonates

In our study, 58.3% of the term neonates showed a PDA at the time of measurement. In the preterm group, 53.6% of the preterm neonates had a PDA. This result is against our expectations, because it is known that the probability of a PDA is higher in preterm neonates.<sup>[21]</sup> The median gestational age of our preterm group with PDA is 33.1 (30.9-35.6) weeks of gestation which shows that no preterm under 30 weeks of gestation is included in this group. Clyman suggests that: *“essentially all healthy preterm infants of 30 weeks’ gestation or greater will have closed their ductus by the fourth day after birth.”*<sup>[21]</sup>

Many of the preterm neonates in this study were accepted at neonatal intensive care unit (NICU) not because of being severely sick, but because of prematurity. The probability of these neonates having a PDA is not as high as for neonates born before the 30<sup>th</sup> week of gestation, or for severely sick neonates.<sup>[21]</sup> On the contrary, term neonates were admitted at NICU because they showed symptoms of respiratory distress syndrome (which delays ductus closure) or raised suspicion of infection. In addition, the validity of the term group is rather low because of the small number of included term neonates (N=9).

In our study, the mean gestational age of the preterm neonates with PDA is significantly lower than in the preterm neonates without PDA. This result underlines the widely accepted hypothesis that the sub-categories of prematurity (very preterm, moderate to late preterm) correlate with the probability of a PDA. The influence of gestational age on peripheral muscle oxygenation is most probably due to gestational age dependent weight/subcutaneous tissue which was not different in the present study.<sup>[85, 120]</sup>

Our data show, that the mean diameter per kg bodyweight was significantly higher in preterm neonates than in term neonates ( $p < 0.001$ ). We conclude from this finding that a PDA in a preterm neonate may be of higher hemodynamic relevance. A limitation of this study is that only the diameter of the PDA, and not the hemodynamic significance, was assessed.

### 4.3.2 Macro- and microcirculatory parameters

Neonates with a PDA had significantly lower  $\text{SaO}_2$  values compared to neonates without a PDA. We also found a statistically significant negative correlation between PDA diameter per kg body weight and  $\text{SaO}_2$ . As mentioned above, the pulse oximeter was always placed post-ductal at the foot.  $\text{SaO}_2$  therefore represents the post-ductal arterial oxygen saturation. Because of the left to right shunting over the PDA, blood is diverted from the systemic circulation back into the pulmonary circulation. Thus, a PDA “steals” blood from the systemic circulation.<sup>[121]</sup> As a result, peripheral blood flow decreases, followed by a natural reduction in peripheral oxygen delivery.<sup>[14]</sup>

Assuming a reduction of oxygen delivery to peripheral tissue, one compensatory mechanism might be the increase of HR. In our study, neonates with a PDA had a significantly higher HR than neonates without a PDA. Our data also show a positive correlation between the diameter of the PDA and the HR.

In order to measure oxygen delivery ( $\text{DO}_2$ ) – representing peripheral perfusion - in a certain tissue, NIRS can be used. In the present study,  $\text{DO}_2$  values tended to be lower in neonates with an open DA but did not differ significantly between groups. The lack of significant differences may be explained by the small number of included neonates and additional factors such as arterial blood pressure and body temperature that influence NIRS measurements.<sup>[84, 85]</sup> For compensation of reduced post ductal blood flow, neonates responded with an increase of the heart rate, correlating with the diameter of the DA.<sup>[120]</sup>

*“If the peripheral tissue metabolic rate and thus  $\text{VO}_2$  is preserved in the face of reduced blood flow there must be a corresponding increase in peripheral FOE.”<sup>[85]</sup>*

Our data showed a significant positive correlation between DA diameter and FOE in the groups of all neonates and in the preterm group. These data in our study are consistent with the results of Kissack CM et al.<sup>[14]</sup> However, it has to be noted that there was a large variation, e.g. the highest value (0.50) was found in a neonate with a closed DA (compare to Figure 29).

$\text{VO}_2$  did not differ significantly between groups. Accordingly, correlation analysis did not show a significant correlation between  $\text{VO}_2$  and DA diameter. Assuming that metabolic rate, and thus, oxygen consumption is preserved, reduced oxygen delivery can be considered to be the reason for an increased FOE in peripheral tissue in preterm

neonates with open DA.<sup>[14]</sup> As there was only a trend to impaired peripheral muscle perfusion, differences in SaO<sub>2</sub> may explain results of FOE. SaO<sub>2</sub> was different between groups and showed a negative association with DA diameter.<sup>[120]</sup>

Factors like birth weight, actual weight, gestational age, heart rate, blood pressure, diameter of calf, and subcutaneous adipose tissue thickness are related to VO<sub>2</sub>.<sup>[84, 85]</sup> In order to rule out other influencing parameters, the limb temperature, and peripheral temperature were measured during the NIRS measurement. Nevertheless, some factors influencing the measurement cannot be changed, and thus their possible influence on the NIRS measurement remains. In adults, several studies have shown that VO<sub>2</sub> increases with increasing physical activity.<sup>[122, 123]</sup> In contrary to adults, it is difficult to examine physical activity under standardized conditions in neonates. Even though the patients were motionless during the time of measurement, we cannot rule out differences in heart rate, alertness, and muscle tone influencing oxygen metabolism and VO<sub>2</sub>.<sup>[85]</sup>

Assuming a reduced oxygen delivery and an increased FOE, one would expect, that the mixed venous oxygenation (SvO<sub>2</sub>) should be reduced, as well. Indeed, our data showed a significant decrease in SvO<sub>2</sub> corresponding to an increase in DA diameter.

Tissue oxygenation index (TOI) represents the oxygen saturation across veins, capillaries, and arteries in a tissue. It is a parameter to assess the cardio circulatory status of a patient at its lowest level – the microcirculation.

Our results showed a significant decrease in peripheral TOI with increasing DA diameter when all 40 neonates were included in the analysis. Since comparison of the two groups (neonates with PDA and neonates without PDA) did not show any significant differences in the demographic and clinical parameters, the decrease in pTOI suggests disturbances in microcirculation in the group of neonates with PDA. However, the correlation is weak; e.g. eliminating just three data points (those with highest DA values in Figure 25) in the set of 40 data would erase the significant correlation, indicating that it can easily happen that no significant correlation can be found in another group of patients. Such a weak correlation does not allow any prediction for the individual child: for instance, the highest value of pTOI (77%) was found at 0.9 mm/kg body weight (Figure 25).

As a result of reduced peripheral blood flow (PBF) and, and thus a decreased perfusion pressure, tissues show a localized vasoconstriction. Shimada et al. pointed out that the heart of a preterm neonate is capable of compensating a cerebral undersupply by increasing the left ventricular output, but is unable to maintain the post ductal blood flow because of decreased perfusion pressure and increased localized vascular resistance.<sup>[122]</sup> After PDA closure, these changes disappear.<sup>[122, 124]</sup> Despite an excessive left-to-right shunt, neonates are capable of increasing their left ventricular output in order to maintain an effective systemic blood flow. Only with left-to-right shunts of more than 50% of left ventricular output, effective systemic blood flow falls.<sup>[21]</sup> Animal model studies have shown that this is true in term animals, but not in preterm animals. Preterm animals were not capable of such a high percentage of compensation.<sup>[21, 36]</sup>

As an additional parameter, cerebral tissue oxygenation index (cTOI) was measured. We did not find a significant correlation between the diameter of the PDA and cTOI. This result is consistent with the results published by Binder-Heschl et al.<sup>[123]</sup> Binder-Heschl et al found a significant negative correlation between the diameter of the PDA and cTOI at the time of the first echocardiography, which was captured on the first day of life. Within their study a second echocardiography was performed after the first day of life. At the time of the second echocardiography they did not find any significant correlation between the diameter of the PDA and cTOI anymore.<sup>[123]</sup> Since in the present study the average age at the time of measurement was 16.6 hours, our data should be compared to the second echocardiography of Binder-Heschl et al.<sup>[123]</sup> Nevertheless, it has to be pointed out that the power of these values is rather low, since the number of neonates with a valid cTOI value is low (N=23).

Disturbances in microcirculation in association with a hemodynamically relevant PDA have been visualized by orthogonal polarization spectral (OPS) imaging and sidestream dark field imaging.<sup>[124]</sup> Hiedl et al. showed that functional vessel density in neonates with a hemodynamically significant PDA was significantly lower compared to neonates with a non-significant PDA. After PDA closure, these differences disappeared again.<sup>[124]</sup> The present results are in accordance with these observations.

## 5 Conclusion

In our group of neonates, peripheral tissue oxygenation index, venous oxygenation saturation, and arterial oxygen saturation decreased with increasing diameter of the DA. Fractional oxygen extraction and heart rate showed an increase with increasing diameter of a PDA.

According to data obtained from our group, an open DA influences peripheral oxygenation parameters in preterm neonates. With increasing DA diameter, the oxygenation of peripheral muscle tissue decreased and, as a consequence oxygen extraction increased in order to compensate for that.

The present study indicates that the diameter of a DA influences peripheral oxygenation and perfusion in neonates. Conclusions for individuals cannot be deduced from the correlations obtained for the groups due to substantial variations in individual measurements. However, the results obtained are consistent and are in line with the physiological expectations. Further studies are necessary to figure out whether the pronounced deviation of some individuals from the significant results found for the group are due to accuracy and reliability limitations of the measurement methods used, or due to differences in the individual physiological behaviour.



## 6 Summary

**Introduction:** The aim of this study was to analyze the effect of a patent ductus arteriosus (PDA) on the microcirculation of neonates. For this purpose in 40 (28 preterm and 12 term) neonates the peripheral muscular microcirculation was investigated using near-infrared spectroscopy (NIRS). A functional echocardiography was performed to measure the diameter of the ductus arteriosus (DA). The 40 neonates were stratified into nine sub-groups taking into account the following stratification parameters: preterm birth, term birth, open, and closed DA.

**Results:** In the group of all 40 neonates, neonates with a PDA had significantly lower arterial oxygen saturation ( $\text{SaO}_2$ ) values than those with a closed DA. This has also been shown in preterm neonates; preterm neonates with a PDA had significantly lower  $\text{SaO}_2$  values than preterm neonates with a closed DA. In the group of all neonates and the preterm group, results showed a significant negative correlation between  $\text{SaO}_2$  values and the DA diameter. The heart rate (HR) was significantly higher in neonates with an open DA, compared to those with a closed DA. A significant positive correlation between HR and DA diameter was found in the group of all neonates, as well as in the preterm group. Fractional oxygen extraction (FOE) values were significantly higher in preterm neonates with a PDA than in those with a closed DA and there was a significant positive correlation between FOE values and the DA diameter. Our results showed a significant decrease in peripheral tissue oxygenation index (pTOI) with increasing DA diameter. In addition, the mixed venous saturation ( $\text{SvO}_2$ ) was lower in neonates with a larger PDA diameter and showed a significant negative correlation with increasing DA diameter.

**Conclusion:** According to the data obtained from our group, an open DA influences peripheral oxygenation parameters in preterm neonates. With increasing DA diameter, the oxygenation of peripheral muscle tissue decreased in the group and, as a consequence, oxygen extraction increased in order to compensate for the undersupply of oxygen. Even though significant correlations were found, the low number of included neonates ( $N=40$ ) as well as the large deviation from the regression line have to be considered. The results are consistent, match the expected physiological pattern, and are in line with study results of other groups.

## 7 Zusammenfassung

**Einleitung:** Das Ziel dieser Studie war es, den Effekt eines persistierenden Duktus Arteriosus (PDA) auf die periphere Perfusion und Oxygenierung bei Neu- bzw. Frühgeborenen zu evaluieren. Dafür wurde bei 40 Neugeborenen (28 Frühgeborene, 12 Reifgeborene) die periphere Perfusion und Oxygenierung mittels einer peripheren Nahinfrarotspektroskopie (NIRS) Messung evaluiert. Eine funktionelle Echokardiographie wurde innerhalb von sechs Stunden vor bis sechs Stunden nach der NIRS-Messung durchgeführt. Die 40 Neugeborenen wurden in Abhängigkeit ihres Gestationsalters und ihres Duktus Arteriosus (offen oder geschlossen) in neun Untergruppen eingeteilt: Alle, Frühgeborene und Reifgeborene, jeweils mit offenem und geschlossenem Duktus Arteriosus.

**Ergebnisse:** Neugeborene mit einem PDA hatten eine signifikant niedrigere arterielle Sauerstoffsättigung ( $\text{SaO}_2$ ) als Neugeborene mit geschlossenem Duktus Arteriosus (DA). Dasselbe Ergebnis konnten wir in der Gruppe der Frühgeborenen zeigen. Die Herzfrequenz (HR) war signifikant höher bei Neugeborenen mit DA als in der Gruppe der Neugeborenen mit geschlossenem DA. Eine signifikante positive Korrelation konnte zwischen der Herzfrequenz und des DA Durchmessers in der Gruppe aller Neugeborenen und auch in der Gruppe der Frühgeborenen, gefunden werden. Die Werte der fraktionellen Sauerstoffextraktion (FOE) waren signifikant höher bei Frühgeborenen mit PDA als bei Frühgeborenen mit geschlossenem DA. Eine signifikante positive Korrelation konnte zwischen FOE Werten und DA Durchmesser gezeigt werden. Des Weiteren konnten unsere Ergebnisse eine Verminderung des peripheren tissue oxygenation index (pTOI) mit zunehmendem DA Durchmesser zeigen. Die Werte der venösen Sauerstoffsättigung ( $\text{SvO}_2$ ) zeigten eine signifikante negative Korrelation mit zunehmendem Durchmesser des DA.

**Schlussfolgerung:** Mit Hilfe von peripher muskulären NIRS Messungen konnte die vorliegende Studie signifikante Unterschiede in der peripheren Perfusion und Oxygenierung bei Neugeborenen mit persistierendem Duktus Arteriosus, im Vergleich zu Neugeborenen mit geschlossenem Duktus Arteriosus, zeigen. Mit größer werdendem Durchmesser des Duktus Arteriosus, verschlechterte sich die Oxygenierung der

peripheren Muskulatur. Als Kompensation erhöhte sich die Sauerstoffextraktion um die Sauerstoff-Minderversorgung zu kompensieren. Obwohl signifikante Korrelationen gefunden wurden, muss die niedrige Fallzahl (N=40) und die große Deviation der Werte um die Regressionslinie berücksichtigt werden. Die Ergebnisse dieser Studie sind in sich konsistent, entsprechen dem erwarteten physiologischen Verhalten und stimmen mit Ergebnissen anderer Forschungsgruppen überein.

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## 9 List of Abbreviations

AHIP	avoiding hypotension in preterm neonates
art.	arterial
ATT	adipose tissue thickness
BNP	brain natriuretic peptide
BP	blood pressure
bpm	beats per minute
cHb	total hemoglobin
CNS	central nervous system
CO <sub>2</sub>	carbon dioxide
COX	cyclooxygenase
CrP	C-reactive protein
cTOI	cerebral tissue oxygenation index
CtOx	cytochrome oxidase
DA	ductus arteriosus
dia.	Diastolic
DO <sub>2</sub>	oxygen delivery
DPF	differential path lengths factor
ECG	electrocardiogram
ELBW	extremely low birth weight
Fig.	figure
FOE	fractional oxygen extraction
Hb	hemoglobin
Hbflow	hemoglobin flow
HbO <sub>2</sub>	oxygenated hemoglobin
HHb	deoxygenated hemoglobin
HR	heartrate
IVH	intraventricular hemorrhage

LDF	Laser Doppler Fluxmetry
LDPI	Laser Doppler Perfusion Imaging
LED	light-emitting diode
LVEF	left ventricular ejection fraction
MAP	mean arterial pressure
Mb	myoglobin
MbO <sub>2</sub>	oxymyoglobin
mmHg	millimeters of mercury
NEC	necrotizing enterocolitis
NICU	neonatal intensive care unit
NIRS	near-infrared spectroscopy
NO	nitric oxide
NT-pro BNP	N terminal prohormone of brain natriuretic peptide
OE	oxygen extraction
OPS	orthogonal polarization spectral imaging
PBF	peripheral blood flow
PDA	patent ductus arteriosus
PG	prostaglandin
PGE <sub>2</sub>	prostaglandin E <sub>2</sub>
pTOI	peripheral oxygenation index
PVH	periventricular hemorrhage
PVL	periventricular leukomalacia
RDS	respiratory distress syndrome
ROP	retinopathy of prematurity
SaO <sub>2</sub>	arterial oxygen saturation
SAT	subcutaneous adipose tissue
SD	standard deviation
SDF	sidestream dark field imaging
SIRS	systemic inflammatory response syndrome
SRS	spatially resolved spectroscopy

SvO <sub>2</sub>	mixed venous oxygen saturation
sys.	systolic
Tab.	Table
TAPSE	tricuspid annular plane systolic excursion
TGF-β	transforming growth factor beta
TOI	tissue oxygenation index
VEGF	vascular endothelial growth factor
ven.	venous
VO <sub>2</sub>	oxygen consumption
WHO	World Health Organization

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## 12 Appendix

### Parents' information and consent

#### *Elterninformation<sup>1</sup> und Einwilligungserklärung zur Teilnahme an der klinischen Studie*

#### **Vermeidung von Blutdruckabfällen bei Frühgeborenen**

Sehr geehrte (werdende) Eltern!

Wir laden Sie ein, dass Ihr neugeborenes Kind an der oben genannten klinischen Studie teilnimmt. Die Aufklärung darüber erfolgt in einem ausführlichen ärztlichen Gespräch.

**Die Teilnahme Ihres Kindes an dieser klinischen Studie erfolgt freiwillig. Sie können jederzeit ohne Angabe von Gründen Ihr Kind aus der Studie ausscheiden lassen. Die Ablehnung der Teilnahme oder ein vorzeitiges Ausscheiden aus dieser Studie hat keine nachteiligen Folgen für die medizinische Betreuung Ihres Kindes.**

Klinische Studien sind notwendig, um verlässliche neue medizinische Forschungsergebnisse zu gewinnen. Unverzichtbare Voraussetzung für die Durchführung einer klinischen Studie ist jedoch, dass Sie Ihr Einverständnis zur Teilnahme an dieser klinischen Studie schriftlich erklären. Bitte lesen Sie den folgenden Text als Ergänzung zum Informationsgespräch mit Ihrem Studienarzt sorgfältig durch und zögern Sie nicht Fragen zu stellen.

Bitte unterschreiben Sie die Einwilligungserklärung nur

- wenn Sie Art und Ablauf der klinischen Studie vollständig verstanden haben,
- wenn Sie bereit sind, der Teilnahme Ihres Kindes zuzustimmen und
- wenn Sie sich über Ihre Rechte als Teilnehmer an dieser klinischen Studie im Klaren sind.

Zu dieser klinischen Studie, sowie zur Patienteninformation und Einwilligungserklärung wurde von der zuständigen Ethikkommission eine befürwortende Stellungnahme abgegeben.

<sup>1</sup> Wegen der besseren Lesbarkeit wird im weiteren Text zum Teil auf die gleichzeitige Verwendung weiblicher und männlicher Personenbegriffe verzichtet. Gemeint und angesprochen sind – sofern zutreffend – immer beide Geschlechter.

### 1. Was ist der Zweck der klinischen Studie?

Niedriger Blutdruck bzw. wiederholte Blutdruckabfälle kommen bei Frühgeborenen vor allem innerhalb der ersten 48 Lebensstunden häufig vor. Diese Kinder haben

somit ein erhöhtes Risiko im Rahmen der Blutdruckabfälle eine Gehirnschädigung zu erleiden. Gründe für diese Blutdruckabfälle sind häufig Infektionen. Erste Zeichen einer Herz- Kreislaufbeeinträchtigung im Rahmen einer Infektion sind bei Frühgeborenen aber schwer zu erkennen.

Zweck dieser klinischen Studie ist es daher, gleichzeitig die Sauerstoffsättigung (Anreicherung von Sauerstoff im Blut) im Gehirn als auch im Muskelgewebe nicht invasiv (ohne die Haut zu verletzen) mit Nah-Infrarotspektroskopie (mit rotem Licht außerhalb des sichtbaren Bereichs) durchgehend innerhalb der ersten 24 Lebensstunden bei mehr als 3 Wochen zu früh geborenen Neugeborenen zu messen, um so zu versuchen, vorzeitige Beeinträchtigungen des Herz-Kreislaufsystems zu erkennen.

Weiters ist es Ziel herausfinden, ob dies eine hilfreiche zusätzliche Überwachung bei intensivgepflegten kleinen Frühgeborenen ist, und ob es möglich ist, durch genau definierte Behandlungsrichtlinien die Blutdruckabfälle zu verringern und somit auch den Verbrauch von kreislaufunterstützenden Medikamenten zu vermindern. In weiterer Folge wollen wir dadurch die möglichen Gehirnschädigungen und die Entwicklung der Frühgeborenen bzw. das Überleben verbessern.

## **2. Wie läuft die klinische Studie ab?**

Diese klinische Studie wird an der Frühgeborenen Station der Universitätsklinik für Kinder- und Jugendheilkunde Graz durchgeführt. Insgesamt werden ungefähr 108 Personen daran teilnehmen.

Vor Aufnahme in diese klinische Studie wird die mütterliche und kindliche Vorgeschichte erhoben. Sollte Ihr Kind ein Frühgeborenes sein (mehr als 3 Wochen vor dem errechneten Geburtstermin geboren), wird es im Falle einer Aufnahme an der Frühgeborenenstation innerhalb der ersten 6 Lebensstunden in die Studie eingeschlossen.

Im Rahmen dieser klinischen Studie wird Ihr Kind mittels Computersystem in eine der folgenden zwei Gruppen zugeteilt:

### Untersuchungsgruppe oder Kontrollgruppe

Untersuchungsgruppe: Innerhalb der ersten 6 Lebensstunden beginnend erfolgt eine für die behandelnden Ärzte sichtbare Überwachung der Sauerstoffsättigungen des Gehirns und des peripheren Muskelgewebes für 24 Stunden. In 6-Stunden Abständen wird das Verhältnis dieser beiden Sättigungen berechnet und bei einer Zunahme von über 5% dieses Verhältnisses wird der behandelnde Arzt folgende Untersuchungen vornehmen:

- Echokardiographie (eine Ultraschalluntersuchung des Herzens)  
neben routinemäßiger Beurteilung

- klinische Einschätzung der Kreislagsituation
- Standardüberwachungen (Blutdruck) und
- bei Beatmung Beurteilung der Beatmungssituation

Unter Berücksichtigung der oben genannten Untersuchungen werden Therapieanpassungen entsprechend genau definierten Behandlungsrichtlinien durchgeführt. Es werden keine Therapien durchgeführt, die nicht auch in der Routinebehandlung von kleinen Frühgeborenen verwendet werden.

Kontrollgruppe: Innerhalb der ersten 6 Lebensstunden beginnend erfolgt eine für die behandelnden Ärzte nicht-sichtbare Überwachung der Sauerstoffsättigungen des Gehirns und des peripheren Muskelgewebes für 24 Stunden. Diese Kinder erhalten eine Behandlung entsprechend der Routine.

Ob diese Überwachung sichtbar ist oder nicht ist zufallsbedingt. Die Wahrscheinlichkeit dass Ihr Kind in einer der beiden Gruppen ist, beträgt 50%:50%. Sollten Sie Zwillinge haben, wird nur das erstgeborene Frühgeborene in die Studie eingeschlossen.

Die Sauerstoffsättigungen im Gehirn und im peripheren Muskelgewebe werden in beiden Gruppen mit der Nah-Infrarotspektroskopie (mit rotem Licht außerhalb des sichtbaren Bereichs) gemessen. Dazu wird jeweils ein Sensor der einen Lichtempfänger und ein zweiter, der eine Lichtquelle enthält auf die Stirn als auch auf den rechten Unterarm aufgelegt und mit einem Verband befestigt. Die Untersuchung mittels Nah- Infrarot-Spektroskopie ist vollkommen schmerzfrei, und das Licht ist für den Körper unbedenklich.

Während der Untersuchung erfolgt mehrmalig das Aufblasen einer Blutdruckmanschette am Oberarm auf einen Druck von 10-20mmHg für zwanzig Sekunden zur Unterbrechung des Blutrückflusses aus dem Unterarm. Mit Hilfe dieser Daten werden in weiterer Folge der Blutfluss und der Sauerstoffumsatz am Unterarm berechnet.

Eine Reihe von Untersuchungen und Eingriffen werden im Zuge der Behandlung von Frühgeborenen durchgeführt, gleichgültig, ob Sie nun an dieser klinischen Studie teilnehmen oder nicht. Diese werden von Ihrem Studienarzt im Rahmen des üblichen ärztlichen Aufklärungsgespräches mit Ihnen besprochen.

Um mögliche Schädigungen des Gehirns zu beurteilen, werden folgende Ultraschallkontrollen, die bei allen Frühgeborenen im Rahmen des stationären Aufenthaltes routinemäßig durchgeführt werden, zu folgenden Zeitpunkten ausgewertet:

- Ultraschall des Kopfes am 1., 2., 4., 7. und 14. Tag nach der Geburt und bei

Entlassung.

Folgende Kontrollen werden ausschließlich aus Studiengründen durchgeführt:

- Echokardiographie in der Untersuchungsgruppe vor und nach einer Therapieanpassung entsprechend den Behandlungsrichtlinien

### **3. Worin liegt der Nutzen einer Teilnahme an der klinischen Studie?**

Mit der Anwendung von Nah-Infrarotspektroskopie kann möglicherweise eine Beeinträchtigung der Herz-Kreislaufsituation vor allem bei Frühgeborenen mit Infektion vorzeitig erkannt und somit mitunter gefährliche Blutdruckabfälle verhindert und in weiterer Folge auch der Einsatz von kreislaufunterstützenden Medikamenten bzw. Organ- und Gehirnschäden verhindert oder verringert werden.

Die Ergebnisse dieser klinischen Studie sollen dazu beitragen, dass für Frühgeborene eine Verbesserung der Überwachung und der Behandlung gefunden wird.

### **4. Gibt es Risiken, Beschwerden und Begleiterscheinungen?**

Nah-Infrarot Spektroskopie ist vollkommen schmerzfrei, und das Licht ist für den Körper unbedenklich. Es können leichte Hautirritationen auftreten, die aber durch regelmäßige Kontrollen und Neuanlage der Sensoren alle 6 Stunden verhindert werden sollen.

Im Rahmen der Hantierung der Geräte kann ihr Kind in seiner Ruhe gestört werden, bzw. können intensivmedizinische Geräte verrutschen - dies kann aber auch bei jedem Routine-Eingriff passieren. Um dieses Risiko zu minimieren werden Gerätebenutzungen bzw. -veränderungen nur von geschultem Personal durchgeführt.

Es wird davon ausgegangen, dass die Anpassung der Therapie zu einer Verhinderung von Blutdruckabfällen und dementsprechend zu einer Stabilisierung der Herz- Kreislaufsituation führt, sollten unvorhersehbare Risiken auftreten werden diese registriert und wenn nötig entsprechende Maßnahmen gesetzt.

### **5. Was ist zu tun beim Auftreten von Symptomen, Begleiterscheinungen und/oder Verletzungen?**

Ihr Kind ist während der Studie in intensivmedizinischer ärztlicher Betreuung und Beobachtung.

## 6. Versicherung

Als Teilnehmer an dieser klinischen Prüfung besteht für Ihr Kind ein Versicherungsschutz, der alle Schäden abdeckt, die an seinem Leben oder seiner Gesundheit durch die an Ihrem Kind durchgeführten Maßnahmen der klinischen Prüfung verursacht werden können, mit Ausnahme von Schäden auf Grund von Veränderungen des Erbmateri als in Zellen der Keimbahn.

Die Versicherung wurde für Ihr Kind bei der:

Wiener Städtischen Allgemeinen Versicherungs AG  
HF 2 Haftpflichtabteilung  
Schottenring 30  
1010 Wien  
Tel.: 050 350  
Polizzennummer: 08-N811 957

Auf Wunsch können Sie in die Versicherungsunterlagen Einsicht nehmen.

Im Schadensfall können Sie sich direkt an den Versicherer wenden und Ihre Ansprüche selbständig geltend machen. Für den Versicherungsvertrag ist österreichisches Recht anwendbar, die Versicherungsansprüche sind in Österreich einklagbar.

Zur Unterstützung können Sie sich auch an die Patientenadvokatur oder Patientenvertretung wenden.

## 7. Wann wird die klinische Studie vorzeitig beendet?

Sie können jederzeit auch ohne Angabe von Gründen, die Teilnahmebereitschaft widerrufen und Ihr Kind aus der klinischen Studie ausscheiden ohne dass Ihrem Kind dadurch irgendwelche Nachteile für die weitere medizinische Betreuung entstehen.

Ihr Studienarzt wird Sie über alle neuen Erkenntnisse, die in Bezug auf diese klinische Studie bekannt werden, und für Sie und Ihr Kind wesentlich werden könnten, umgehend informieren. Auf dieser Basis können Sie dann Ihre Entscheidung zur **weiteren** Teilnahme Ihres Kindes an dieser klinischen Studie neu überdenken.

Es ist aber auch möglich, dass Ihr Studienarzt entscheidet, die Teilnahme Ihres Kindes an der klinischen Studie vorzeitig zu beenden, ohne vorher Ihr Einverständnis einzuholen. Die Gründe hierfür können sein:

a) Ihr Kind entspricht nicht den Erfordernissen der klinischen Studie;

b) Ihr Studienarzt hat den Eindruck, dass eine weitere Teilnahme an der klinischen Studie nicht im Interesse Ihres Kindes ist

**8. In welcher Weise werden die im Rahmen dieser klinischen Studie gesammelten Daten verwendet?**

Sofern gesetzlich nicht etwas anderes vorgesehen ist, haben nur die Studienärzte und deren Mitarbeiter Zugang zu den vertraulichen Daten, in denen Sie namentlich genannt werden. Diese Personen unterliegen der Schweigepflicht.

Die Weitergabe der Daten erfolgt ausschließlich zu statistischen Zwecken und Sie werden ausnahmslos nicht namentlich genannt. Auch in etwaigen Veröffentlichungen der Daten dieser klinischen Studie werden Sie nicht namentlich genannt.

**9. Entstehen für die Teilnehmer Kosten? Gibt es einen Kostenersatz oder eine Vergütung?**

Durch die Teilnahme Ihres Kindes an dieser klinischen Studie sind für Sie keine zusätzlichen Kosten zu erwarten. Da sich Ihr Neugeborenes während der Studie bereits in stationärer Behandlung befindet, sind somit keine Vergütungen vorgesehen.

**10. Möglichkeit zur Diskussion weiterer Fragen**

Für weitere Fragen im Zusammenhang mit dieser klinischen Studie stehen Ihnen Ihr Studienarzt und seine Mitarbeiter gern zur Verfügung. Auch Fragen, die Ihre Rechte und die Rechte Ihres Kindes als Patient und Teilnehmer an dieser klinischen Studie betreffen, werden Ihnen gerne beantwortet.

Name der Kontaktperson: Ass. Prof. Dr Pichler Gerhard  
Ständig erreichbar unter: 0316 385 80520

Name der Kontaktperson: Univ. Prof. Dr Berndt Urlesberger  
Ständig erreichbar unter: 0316 385 81133

**11. Einwilligungserklärung**

Name des Patient in Druckbuchstaben:.....  
Geb.Datum: ..... Code:.....

Ich erkläre mich bereit, an der klinischen Studie „*Vermeidung von Blutdruckabfällen bei Frühgeborenen*“ teilzunehmen.

Ich bin von Herrn/Frau (*Dr.med.*) ..... ausführlich und verständlich über die *Nah-Infrarot-Spektroskopie*, mögliche Belastungen und Risiken, sowie über Wesen, Bedeutung und Tragweite der klinischen Studie, die bestehende Versicherung sowie die sich für mich und mein Kind daraus ergebenden Anforderungen aufgeklärt worden. Ich habe darüber hinaus den Text dieser Patientenaufklärung und Einwilligungserklärung, die insgesamt 6 Seiten umfasst, gelesen. Aufgetretene Fragen wurden mir vom Studienarzt verständlich und genügend beantwortet. Ich hatte ausreichend Zeit, mich zu entscheiden. Ich habe zurzeit keine weiteren Fragen mehr.

Ich werde den ärztlichen Anordnungen, die für die Durchführung der klinischen Studie erforderlich sind, Folge leisten, behalte mir jedoch das Recht vor, die freiwillige Mitwirkung meines Kindes jederzeit zu beenden, ohne dass ihm daraus Nachteile für seine weitere medizinische Betreuung entstehen.

Ich bin zugleich damit einverstanden, dass meine und die meines Kindes im Rahmen dieser klinischen Studie ermittelten Daten aufgezeichnet werden. Um die Richtigkeit der Datenaufzeichnung zu überprüfen, dürfen Beauftragte der zuständigen Behörden beim Studienarzt Einblick in meine personenbezogenen Krankheitsdaten nehmen.

Die Bestimmungen des Datenschutzgesetzes in der geltenden Fassung werden eingehalten.

Eine Kopie dieser Patienteninformation und Einwilligungserklärung habe ich erhalten. Das Original verbleibt beim Studienarzt.

.....  
(Datum und Unterschrift des Patienten)

.....  
(Datum, Name und Unterschrift des verantwortlichen Arztes)

***(Der Patient erhält eine unterschriebene Kopie der Patienteninformation und Einwilligungserklärung, das Original verbleibt im Studienordner des Studienarztes)***



## **Elterninformation und Einwilligungserklärung zur Teilnahme an der klinischen Prüfung**

### **Der Einfluß von hämodynamischen Parametern (Kreislaufwerten / Blutdruck) auf die zerebrale Oxygenierung (Sauerstoffgehalt des Gehirns) bei beatmeten Frühgeborenen während des ersten Lebenstages.**

#### **Sehr geehrte Mutter, sehr geehrter Vater!**

Wir laden Sie ein, dass ihr Neugeborenes an der oben genannten klinischen Prüfung teilnimmt. Die Aufklärung darüber erfolgt in einem ausführlichen ärztlichen Gespräch.

**Die Teilnahme Ihres neugeborenen Kindes an dieser klinischen Prüfung erfolgt freiwillig. Sie können jederzeit ohne Angabe von Gründen aus der Studie ausscheiden. Die Ablehnung der Teilnahme oder ein vorzeitiges Ausscheiden aus dieser Studie hat keine nachteiligen Folgen für Ihre medizinische Betreuung und die medizinische Betreuung Ihres Neugeborenen.**

Klinische Prüfungen sind notwendig, um verlässliche neue medizinische Forschungsergebnisse zu gewinnen. Unverzichtbare Voraussetzung für die Durchführung einer klinischen Prüfung ist jedoch, dass Sie Ihr Einverständnis zur Teilnahme ihres Neugeborenen Kindes an dieser klinischen Prüfung schriftlich erklären. Bitte lesen Sie den folgenden Text als Ergänzung zum Informationsgespräch mit Ihrem Arzt sorgfältig durch und zögern Sie nicht Fragen zu stellen.

Bitte unterschreiben Sie die Einwilligungserklärung nur

- wenn Sie Art und Ablauf der klinischen Prüfung vollständig verstanden haben,
- wenn Sie bereit sind, der Teilnahme Ihres Neugeborenen zuzustimmen und
- wenn Sie sich über die Rechte als Teilnehmer an dieser klinischen Prüfung im Klaren sind.

Zu dieser klinischen Prüfung, sowie zur Elterninformation und Einwilligungserklärung wurde von der zuständigen Ethikkommission eine befürwortende Stellungnahme abgegeben.

#### **1. Was ist der Zweck der klinischen Prüfung?**

Der Zweck dieser klinischen Prüfung ist es, die Sauerstoffsättigung (Sauerstoffgehalt des Blutes) in Gehirn und Muskel bei reifen Neugeborenen und Frühgeborenen mit und ohne arterieller Hypotonie (zu niedriger Blutdruck) über 24 Stunden (beginnend innerhalb der ersten sechs Lebensstunden) zu messen und zu vergleichen, um eventuelle Unterschiede feststellen zu können.

Die Untersuchung der peripher muskulären Sauerstoffsättigung bzw. der Sauerstoffsättigung im Gehirn erfolgt mit einem „Nahinfrarotspektroskopiegerät“ (INVOS), wobei je eine Lichtquelle und ein Lichtempfänger (Sensoren) am rechten Unterarm bzw. am linken Unterschenkel und zwei auf die Stirn aufgelegt werden. Die Sensoren werden mit einem Verband befestigt.

Es werden Veränderungen des roten Blutfarbstoffes, der der Träger des Sauerstoffes im Blut ist, gemessen, indem Licht in das Gewebe gestrahlt wird und die Veränderungen des Lichtstrahls gemessen werden. Die Untersuchung mittels Nahinfrarotspektroskopie ist vollkommen schmerzfrei, und das Licht ist für den Körper unbedenklich.

Zweimal wird die peripher muskuläre Durchblutung mit „Nahinfrarotspektroskopie“ gemessen, wobei je eine Lichtquelle und je ein Lichtempfänger am linken Unterschenkel bzw. am rechten Unterarm aufgelegt wird und mit einem Verband befestigt wird. Während dieser Untersuchung erfolgt mehrmalig (3x) das Aufblasen einer Blutdruckmanschette am Oberarm und Oberschenkel auf einen Druck von 10-20mmHg für zwanzig Sekunden zur Unterbrechung des Blutrückflusses aus dem Unterarm bzw. Unterschenkel. Mit Hilfe dieser Daten werden in weiterer Folge der Blutfluss und der Sauerstoffumsatz an der Unterarm- und Unterschenkelmuskulatur berechnet.

Zusätzlich wird 2-mal ein Ultraschall des Herzens und des Kopfes gemacht.

## **2. Welche anderen Messmöglichkeiten gibt es?**

Zur Messung der Sauerstoffsättigung und Durchblutungsparameter in Gehirn und Muskel stellt die Nah-Infrarot Spektroskopie die einzige Möglichkeit dar.

## **3. Wie lange dauert die Studie?**

Die Untersuchungen werden innerhalb der ersten 24- (30) Lebensstunden durchgeführt.

Die Messdauer beträgt 24 Stunden.

## **4. Wie läuft die klinische Prüfung ab?**

Diese klinische Prüfung wird während des stationären Aufenthaltes Ihres Neugeborenen an der Abteilung für Neonatologie der Univ. Klinik für Kinder und Jugendheilkunde durchgeführt, und es werden insgesamt ungefähr 264 Neugeborene daran teilnehmen.

Vor Aufnahme in diese klinische Prüfung werden wichtige Daten Ihrer Schwangerschaft und Ihres Neugeborenen erhoben.

Innerhalb der ersten 6 Lebensstunden werden die Lichtquellen/-empfänger zu kontinuierlichen Sauerstoffgehalts-Messungen (Nahinfrarotspektroskopie) am Kopf und am Bein bzw. Arm mit einem Verband angebracht und für 24 Stunden belassen.

Routinemäßig werden der Sauerstoffgehalt des Blutes und der Puls mit Pulsoximetrie gemessen. Ein „Pulsoximeter“ ist ein Gerät, das durch Veränderungen von Licht beim Durchtritt durch Gewebe den Sauerstoffgehalt des Blutes und den Puls misst. Dieses wird an der Hand oder am Fuß befestigt.

Routinemäßige werden bei ihrem Kind weiters die Herzfrequenz, der Blutdruck, die periphere und rektale Temperatur, die Rekapillierungszeit und falls ihr Kind beatmet wird die Beatmungsparameter gemessen.

Am Beginn und Ende des ersten Lebenstages wird bei ihrem Kind das Herz und das Gehirn mit einem Ultraschallgerät untersucht.

Im Rahmen einer routinemäßigen Blutabnahme werden zusätzlich Marke bestimmt, die Rückschlüsse auf die Herzleistung und die Sauerstoffversorgung des Gehirns geben können.

**Folgende Maßnahmen werden ausschließlich aus Studiengründen durchgeführt:**

Ausschließlich aus Studiengründen werden die Messungen der Sauerstoffsättigung des Gehirns und des Muskels mittels Nahinfrarotspektroskopie und des Herzultraschall durchgeführt.

**5. Was ist Nah-Infrarot Spektroskopie**

Nah-Infrarot Spektroskopie ist ein medizinisches Verfahren, das bereits weitreichend bei Neugeborenen angewandt wurde, und eine Messung der Sauerstoffsättigung des Gewebes ohne Verletzung der Haut (nicht-invasiv) ermöglicht. Nebenwirkungen wurden bisher keine beschrieben.

**6. Worin liegt der Nutzen einer Teilnahme an der Klinischen Prüfung?**

Es ist nicht zu erwarten, dass Ihr Neugeborenes aus der Teilnahme an dieser klinischen Prüfung gesundheitlichen Nutzen ziehen wird.

Die Ergebnisse dieser klinischen Prüfung sollen dazu beitragen, den Zusammenhang zwischen Faktoren des Herz- Kreislaufsystems und der Sauerstoffversorgung in Gehirn und Muskel zu untersuchen um damit in Zukunft, bei anderen Neugeborenen, früher die Notwendigkeit therapeutischer Maßnahmen erkennen zu können.

## **7. Gibt es Risiken, Beschwerden und Begleiterscheinungen?**

Es sind keine Risiken, Beschwerden und Begleiterscheinungen zu erwarten.

## **8. Wann wird die klinische Prüfung vorzeitig beendet?**

Sie können jederzeit, auch ohne Angabe von Gründen, die Teilnahme Ihres Neugeborenen widerrufen und aus der klinischen Prüfung ausscheiden ohne dass Ihnen dadurch irgendwelche Nachteile für die weitere medizinische Betreuung entstehen.

Ihr Prüfarzt wird Sie über alle neuen Erkenntnisse, die in Bezug auf diese klinische Prüfung bekannt werden, und für Sie oder Ihr Neugeborenes wesentlich werden könnten, umgehend informieren. Auf dieser Basis können Sie dann Ihre Entscheidung zur weiteren Teilnahme an dieser klinischen Prüfung neu überdenken.

Es ist aber auch möglich, dass Ihr Prüfarzt entscheidet, die Teilnahme Ihre Neugeborenen an der klinischen Prüfung vorzeitig zu beenden, ohne vorher Ihr Einverständnis einzuholen. Ein Grund dafür kann eine zu starke Unruhe des Neugeborenen sein.

## **9. In welcher Weise werden die im Rahmen dieser klinischen Prüfung gesammelten Daten verwendet?**

Sofern gesetzlich nicht etwas anderes vorgesehen ist, haben nur die Prüfer/innen und deren Mitarbeiter/innen Zugang zu den vertraulichen Daten, in denen Sie oder Ihr Neugeborenes namentlich genannt werden („personenbezogene“ Daten). Weiters können Beauftragte von in- und ausländischen Gesundheitsbehörden, der zuständigen Ethikkommission, sowie – wenn zutreffend – des Auftraggebers der klinischen Prüfung Einsicht in diese Daten nehmen, um die Richtigkeit der Aufzeichnungen zu überprüfen. Diese Personen unterliegen einer gesetzlichen Verschwiegenheitspflicht.

Die Weitergabe der Daten im In- und Ausland erfolgt ausschließlich zu statistischen Zwecken in verschlüsselter (nur „indirekt personenbezogener“) oder anonymisierter Form. Das heißt, Sie und ihr Neugeborenes werden nicht namentlich genannt. Auch in etwaigen Veröffentlichungen der Daten dieser klinischen Prüfung werden Sie und ihr Neugeborenes nicht namentlich genannt.

Die Prüfer/innen und ihre Mitarbeiter/innen unterliegen im Umgang mit den Daten den Bestimmungen des österreichischen Datenschutzgesetzes 2000 in der jeweils geltenden Fassung.

Wenn Sie Ihre Einwilligung zurückziehen und damit die Teilnahme vorzeitig beenden, werden keine neuen Daten mehr über Sie oder ihr Neugeborenes erhoben. Auf Grund gesetzlicher Dokumentationspflichten kann jedoch weiterhin für einen gesetzlich festgelegten Zeitraum eine Einsichtnahme in die personenbezogenen Daten zu Prüfzwecken durch autorisierte, zur Verschwiegenheit verpflichtete Personen erfolgen.

**10. Entstehen für die Teilnehmer Kosten? Gibt es einen Kostenersatz oder eine Vergütung?**

Nein

**11. Möglichkeit zur Diskussion weiterer Fragen**

Für weitere Fragen im Zusammenhang mit dieser klinischen Prüfung stehen Ihnen Ihr Prüfarzt und seine Mitarbeiter gern zur Verfügung. Auch Fragen, die die Rechte als Patient/Eltern und Teilnehmer an dieser klinischen Prüfung betreffen, werden Ihnen gerne beantwortet.

Name der Kontaktperson: PD Dr Pichler Gerhard.....  
Ständig erreichbar unter: 0316 385 80520.....

Name der Kontaktperson: Univ Prof Dr Urlesberger Berndt.....  
Ständig erreichbar unter: 0316 385 81133.....

**12. Einwilligungserklärung**

Name des Patienten in Druckbuchstaben:.....  
Geb.Datum:..... Code: .....

Ich erkläre mich bereit, dass mein Neugeborenes an der klinischen Prüfung:

**„Der Einfluß von hämodynamischen Parametern (Kreislaufwerten / Blutdruck) auf die zerebrale Oxygenierung (Sauerstoffgehalt des Gehirns) bei beatmeten Frühgeborenen während des ersten Lebenstages“**

teilnimmt.

Ich bin von Herrn/Frau (*Dr.med.*) ..... ausführlich und verständlich über die Nah- Infrarot Spektroskopie, mögliche Belastungen und Risiken, sowie über Wesen, Bedeutung und Tragweite der klinischen Prüfung, sowie die sich für mein Neugeborenes daraus ergebenden Anforderungen aufgeklärt worden. Ich habe darüber hinaus den Text dieser Elterninformation und Einwilligungserklärung, die insgesamt 5 Seiten umfasst gelesen. Aufgetretene Fragen wurden mir vom Prüfarzt verständlich und genügend beantwortet. Ich hatte ausreichend Zeit, mich zu entscheiden. Ich habe zurzeit keine weiteren Fragen mehr. Ich werde den ärztlichen Anordnungen, die für die Durchführung der klinischen Prüfung erforderlich sind,

Folge leisten, behalte mir jedoch das Recht vor, die freiwillige Mitwirkung meines Neugeborenen jederzeit zu beenden, ohne dass uns daraus Nachteile für unsere weitere medizinische Betreuung entstehen.

Ich bin zugleich damit einverstanden, dass die im Rahmen dieser klinischen Prüfung ermittelten Daten gespeichert werden. Mir ist bekannt, dass zur Überprüfung der Richtigkeit der Datenaufzeichnung Beauftragte der zuständigen Behörden, der Ethikkommission und ggf. des Auftraggebers beim Prüfarzt Einblick in unsere personenbezogenen Krankheitsdaten nehmen dürfen. Beim Umgang mit den Daten werden die Bestimmungen des Datenschutzgesetzes 2000 beachtet. Eine Kopie dieser Elterninformation und Einwilligungserklärung habe ich erhalten. Das Original verbleibt beim Prüfarzt.

.....  
(Datum und Unterschrift der Eltern)

.....  
(Datum, Name und Unterschrift des verantwortlichen Arztes)

(Die Eltern erhalten eine unterschriebene Kopie der Elterninformation und Einwilligungserklärung, das Original verbleibt im Studienordner des Prüfarztes.)

## 13 Acknowledgements

My special thanks goes to Prof. Berndt Urlesberger and Prof. Orsolya Genzel-Boroviczeny for giving me the opportunity of realizing my thesis at the Department for Neonatology at the University Graz and for designing this cooperative thesis project.

I would like to thank Professor Gerhard Pichler for familiarizing me with near-infrared spectroscopy and for introducing me to the scientific work in the field of neonatology. I am grateful for your support throughout my time at the neonatal ward and for providing me with the opportunity to get a very good insight into neonatology as well as into how to conduct clinical studies. Thank you for your support and patience.

I would like to express my gratitude to Dr. Lukas Peter Miledner for supporting me throughout the whole project.

My special thanks goes to Evelyn Ziehenberger for introducing me to the measuring technique and for her support.

Thank you for the great acceptance in the working group and in the whole team of the neonatal ward.

Last but not least, I would like to thank my family. Thank you, Mama and Papa, for always supporting my ideas and your never ending love. Romy and Tom, thank you for being the best siblings I can imagine.

Thank you, Julie, for being the best friend I ever had. I want to thank you for your endless support, motivation, and friendship through the many years we shared together. I am grateful to have known you.